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The Effect of Cooking Methods on the Quality of Refrigerated and Frozen Whole Crawfish Procambarus Clarkii Girard and Procambarus Zonangulus

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THE EFFECT OF COOKING METHODS ON THE QUALITY OF REFRIGERATED AND FROZEN WHOLE CRAWFISH *PROCAMBARUS CLARKII GIRARD* AND *PROCAMBARUS ZONANGULUS*

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ABSTRACT

Louisiana is famous for its diverse food, culture, and festivals. Crawfish are synonymous with Louisiana cuisine. Approximately 90% of the crawfish harvested in the United States are harvested in Louisiana, but their availability is seasonal (NOAA 2014). Both in-state and out-of-state demand for whole cooked crawfish and for their availability outside the normal harvest season has increased. There is interest from crawfish processors on whether or not steaming of crawfish results in a higher yield than boiling, as traditionally done. This study measures yield, quality parameters, and shelf life of whole boiled and steamed crawfish held in either refrigerated or frozen storage. Microbiological, lipid oxidation, proximate analyses, pH, texture properties, color, mineral, and fatty acid analyses and yield determinations were performed.

Live crawfish (*Procambarus clarkii*), were either boiled or steamed, then stored for up to 11 days in refrigerated (3°C) conditions or six months in frozen storage (-18°C). The crawfish were then peeled and aerobic plate counts and E.coli/coliform counts determined using 3M™ Petrifilms. Lipid oxidation was measured by TBARS analyses. Texture as measured by peak force and work of shearing was determined using a 5-blade Kramer shear attachment on a TA-XT Plus Texture Analyzer. Proximate and pH analyses were conducted using AOAC procedures. Color was evaluated based on Hunter color scale values (L*, a*, and b*) with 10 replications. Mineral analysis was conducted via inductively coupled plasma optical emission spectroscopy (ICP-OES). Fatty acid analysis was conducted using gas chromatography coupled with mass spectrometry (GC-MS) in duplicate. Statistics were conducted using SAS 9.3 with GLM and LS-Mean separation.

Results from these analyses suggest there is no benefit to cooking crawfish via steam versus the boiling method. No appreciable yield difference was observed between the two cooking methods and the shelf life through refrigerated and frozen storage was statistically the same for both cooking methods.

CHAPTER 1: INTRODUCTION

Louisiana is the "Crawfish State" and is responsible for 90 % of the crawfish harvested in the United States (NOAA 2014). Crawfish are a staple of Cajun and south Louisiana culture. To people across the United States, the term crawfish is synonymous with Louisiana (Louisiana Sea Grant 1993). Crawfish epitomize the interesting foods and culture that make Louisiana unique. They are a part of life during the season (spring) in which they are available, especially in south Louisiana. Many social events are centered around crawfish boils. Friends and family get together and boil live crawfish utilizing techniques and seasoning passed down from one generation to the next - everyone's family recipe is the best. By its very nature, the crawfish boil requires whole crawfish because it takes time to peel which makes dining a social event with time for visiting. The opportunity for socializing and enjoying food is something that many Louisianans hold dear and look forward to every year.

Historically, crawfish have been a very regional product and largely consumed in areas near the harvest. Over the last ten to twenty years, there has been an increased interest in crawfish outside of the region where crawfish are harvested. The interest in, and popularity of, "Cajun" cuisine that features crawfish is steadily growing (Davis 1993). Because of this demand, processors have been interested in extending the seasonal availability, increasing storage capabilities, and introducing new ways of offering crawfish to the national and global market.

Louisiana State University's seafood specialist was approached by local processors to investigate ways in which crawfish could be marketed differently and to determine yield differences between steaming and boiling crawfish. The seasonal average yield of tail meat from Louisiana crawfish is around 15% (Romaire and others 2005). Some processors claimed to have heard of 28 to 30% yields with steam cooking. Thus, it was decided to investigate if steaming

would contribute to increased yield of peeled crawfish tail meat. In addition, processors wanted to be able to freeze whole crawfish after cooking so that they could be thawed and peeled in the off-season, but were unsure of the quality of the crawfish meat after extended frozen storage. They also wanted to know the quality of whole crawfish after extended storage. Acceptable quality would allow extended shipment without the losses associated with the shipment of live crawfish during which many perish and assist in the extension of the crawfish-eating season.

As a consequence, objectives of this study were to 1) boil and steam live crawfish and determine their respective yields through eleven days of refrigerated storage and through six months of frozen storage and, 2) proximate analyses, texture, color, microbiological assay, minerals, fatty acid, and oxidation determinations throughout storage to determine the quality of both the boiled and steamed crawfish through refrigerated and frozen storage.

CHAPTER 2: REVIEW OF RELATED LITERATURE

2.1 Background and History of the Crawfish Industry in Louisiana

There is some ambiguity as to the origin of the name crawfish and who was the first to name these animals. According to Horst (2010), Thomas Say in 1817 was the first American scientist to study and call the creatures "crawfish". Jerry Walls (2009), suggested that the naturalist Constatine Fafinesque coined the name in the same year. Thus, the term crawfish predates the term "crayfish" devised by the British scientist Thomas Huxley roughly 50 years later in a widely used 1880 textbook, *The Crayfish, An Introduction to the Study of Zoology* (Walls 2009). The term "crawfish" is a term used by denizens of South Louisiana as well as nicknames such as "mudbugs" and "crawdads". However, in other parts of the country and in scientific papers from around the globe, crawfish are generally called crayfish (Horst 2010). The term crawfish has been credited as correct in scientific writings, and the term occurs almost as frequently in publications as the term crayfish. For simplicity, throughout the rest of this thesis, the term used will be crawfish.

There are approximately 300 species of crawfish native to North America; Louisiana is home to more than three dozen distinct species and subspecies that range in size from less than an inch to over five inches in length (Hobbs 1974; Walls 2009). However, there are only two species both of the *Procambarus* genus that are commercially important, *Procambarus clarkii*, the red swamp crawfish, and *Procambarus zonangulus*, the white river crawfish (Marshall and others 1988; Davis 1994; Romaire and others 2004). In the scientific community, the term 'crawfish' is believed to be the most common name for the species of the genus *Procambarus,* to which the red swamp crawfish and white river crawfish belong (Penn 1943). Both species grow in Louisiana's fresh to brackish waters, in swamps, lagoons, streams, bayous, and even ditches.

One area of Louisiana where crawfish species do not flourish is in piney-woods environments (Penn 1943). The habitat and terrain that red swamp and white river crawfish prefer make them ideally suited to grow prolifically in the Atchafalaya river basin of south Louisiana (Lovell 1968). Figure 1 shows the state of Louisiana with prime crawfish habitat areas delineated. The dotted/shaded area is the area from which the majority of the crawfish harvest occurs. The Atchafalaya river basin constitutes the majority of this region.

Figure 1. Main Crawfish Producing Area in Louisiana (Lovell 1968).

Red swamp crawfish are the predominant species on most crawfish farms and in the southern Louisiana habitat. White river crawfish predominate in northern Louisiana and in Mississippi. However, both species typically occur mixed in the environment and are harvested together in differing amounts with the red swamp crawfish generally dominating the total harvest (Horst 2010). Both species exceed three inches in length as adults. The red swamp crawfish is more tolerant of poor water conditions and warmer temperatures than the white river crawfish (Davis 1994). Distinct physical characteristics allow easy differentiation of the mature animals of

the two species, but these characteristics are not as prevalent when the crawfish are immature which makes immature crawfish more difficult to identify. White river crawfish have narrower and longer pincers while pincers of red swamp crawfish are broader and considerably larger. Immature crawfish of both species closely resemble each other, but mature red swamp crawfish are a deeper and more brilliant red color while mature white river crawfish are paler red in appearance (Horst 2010).

The red swamp crawfish usually dominates and because of their preferred appearance are more desired by processors and consumers (Marshall and others 1988; McClain and Romaire 2004). The flavor of the tail meat is relatively similar between the two species. The hepatopancreas or 'fat' of the red crawfish is a bold orange color with a rich flavor while white crawfish hepatopancreas has a tendency to be a more unappealing green color and is slightly less tasty (Horst 2010). Also, the visual appearance of the cooked crawfish is quite different. The shell of the red swamp crawfish is a brilliant red color when cooked while the white river crawfish has some red coloring but is much paler. For aesthetic and flavor reasons, consumers and processors prefer the red swamp crawfish to the white river crawfish (Marshall and others 1988; Horst 2010)

2.1.1 Economic Impact of Crawfish on the State of Louisiana

The state of Louisiana is the number one producer of crawfish in the United States. Each year roughly 80 million pounds of crawfish are harvested from the combined outputs of both aquaculture and commercial wild caught fisheries (Romaire and others 2005; Walls 2010). This output ranges each year due to weather and water conditions as previously mentioned. The crawfish harvest from Louisiana represents 95% of the domestic crawfish crop and in recent years more than 80% of the domestic harvest has come from aquaculture (Romaire and others

2005). Louisiana has 90% of the United State's crawfish farmers and 99% of its wild crawfish harvesters (Horst and Roberts 1993). Louisiana is a large supplier in the crawfish market, which has large importance to the state economically and culturally.

Crawfish consumption in Louisiana has been recorded as far back as 1793 when fishermen would use a wooden stick with bait tied to one end and simply lift the bait out of the water at 15 minute intervals to see if a crawfish had clung to the bait (Horst 2010). The first recorded commercial catch of crawfish in the United States occurred in 1880. The harvest had a weight of 23,400 pounds and a value of $2,140.00 (Penn 1943). Crawfish developed a presence in seafood markets in the 1800's similarly to lobster on the East Coast, crab on the West Coast, and shrimp on the Gulf (Pitre 1993). As reported by the U.S. Fish Commission in 1893 Crawfish harvests of 1889 and 1890 were 138,000 and 140,650 pounds, respectively (Horst 2010).

Crawfish harvesters and processors want to extend the market outside of Louisiana and the nearby regions. Crawfish tail meat from the same species (*Procambarus clarkii*) harvested in Louisiana has been imported into the U.S. by the Chinese. The influx of Chinese crawfish and crawfish products over the recent decades has caused a number of crawfish processing facilities in Louisiana to close since the Chinese products are cheaper than the Louisiana crawfish (Horst 2010). The harvesters and processors of Louisiana have attempted to market Louisiana crawfish as a local and superior product to the imported Chinese product, but the processors are struggling to identify other avenues to market crawfish.

2.1.2 Availability, Forms, and Processing of Crawfish Products

Crawfish are seasonal and the harvest period stretches from as early as November to as late as August depending on water, temperature, and other natural conditions. Farm-raised crawfish generally appear on the market first, around late November due to the controlled

flooding of ponds in September and October. Wild caught crawfish are not available until later in the season because flooding in the Atchafalaya Basin does not usually occur until December. Also, the Atchafalaya Basin water temperatures are generally lower in December and January than farmed ponds, resulting in slower growth and availability of wild caught crawfish (Horst 2010). The peak season for crawfish harvesting takes place during March through June. During the crawfish season, most of the crawfish harvested domestically are consumed in Louisiana and surrounding areas that have limited crawfish production and processing such as Texas, the Mississippi Gulf Coast, and the Florida panhandle (Romaire and others 2005).

Traditionally the commercially available forms of crawfish were either live or boiled, whole crawfish. Starting in the early to mid 1960's processing and peeling plants began (Lovell 1968) boiling or steaming crawfish with unseasoned water until completely cooked before hand peeling, packaging, and then distributing them either refrigerated or frozen. This allowed meeting the demand of consumers who do not want to peel crawfish themselves. The peeled tail meat is used in dishes such as crawfish etouffee, crawfish bisque, fried crawfish po'boys, and other products (Lovell 1968). Crawfish products such as crawfish cakes and crawfish boudin are also produced. These prepared and typically frozen crawfish products account for only a small portion of the total sales in the crawfish industry but they have helped expand the distribution of crawfish and the markets for value added crawfish products (Romaire and others 2005).

2.1.3 Crawfish Harvesting

The two sources of commercial crawfish in Louisiana are 1) naturally flooded areas including marshes, the Atchafalaya River Basin, lakes, swamps, bayous and canals as mentioned previously and 2) managed production/aquaculture in rice fields and man-made ponds. Crawfish thrive in the extensive wetlands of the lower Mississippi river floodplain which experiences

seasonal flooding and drying. The most productive natural source for crawfish is the Atchafalaya Basin, or spillway (Lovell 1968). The wild crawfish catch can be unpredictable, especially in the Atchafalaya River Basin, due to seasonal variation.

In earlier times, fishermen caught wild crawfish using nets constructed out of cotton webbing held open with a wire frame. Fishermen would use chicken necks, chicken backs, and other bony, cheap and bloody bait typically tied up the center of the net to attract the crawfish. These nets were labor intensive because they had to be closely monitored and harvested frequently to prevent crawfish escaping (Horst 2010). Today, wild crawfish are caught in the traditional pillow trap that are about 0.9 meters (m) long and 0.45 m wide with a funnel entrance that makes it easy for crawfish to get in the trap but difficult to escape (Walls 2009). Traps are baited and collected in the same manner for commercially wild caught and farm raised crawfish. However, the predominant type of trap used for harvesting farm-raised crawfish is a barrel-shaped wire trap called a stand-up trap or a "pop bottle" trap and these contain two to three funnel openings at the base of the trap rather than one like the pillow traps (Walls 2009; Horst 2010).

According to McClain and Romaire (2004), traps are set and collected two to seven days a week, to meet the demand for crawfish during the heart of the season (March through June). This not only maintains a steady supply of crawfish for the market, but it is also beneficial for the crawfish population. When crawfish populations are too dense, they will have a tendency to prey on one another. Crawfish are aggressive creatures and cannibalism as well as non-predatory mortality are often more important in controlling a crawfish population than predation (Huner and others 1978). Crawfish are opportunistic and cannibalism most often occurs on freshly molted individuals whose shells are softer which makes them vulnerable to attack (Mason 1963;

Hutton 1963). Another factor determining the abundance of crawfish is the quality of the water in which they reside. The amount of dissolved oxygen in their water should be higher than three ppm at all times. There should not be too much dead vegetation because this has a tendency to deplete oxygen in the water even though the decaying plant matter serves as food for aquatic invertebrates that are food for the crawfish (McClain and Romaire 2004). Calcium is used in hardening crawfish shells, if the water is too soft, calcium is added in order to keep the habitat more conducive to raising crawfish (Davis 1994).

Weather conditions affect the season for aquacultured crawfish, so the ability to control water conditions greatly reduces the variability of the season. The idea of aquaculture is to construct earthen ponds, which are flooded and dried to mimic the natural occurrences in which crawfish are the most productive (McClain and Romaire 2004). In the 1950's crawfish farming was a modest enterprise, but grew greatly in the 1960's when 10,000 acres of land were devoted to crawfish farming, increasing to 44,000 acres by the mid1970's (Horst 2010). As of 2007, over 160,000 acres were devoted to crawfish farming in Louisiana. Aquaculture has grown to the point that it is responsible for the majority of crawfish produced (estimated 81%) (McClaine and Romaire 2004; Romaire and others 2005; Horst 2010).

2.1.4 Crawfish Processing

Crawfish processing has become a large part of the crawfish industry. Crawfish processing is a modern industry that produces a high quality product available for consumption world-wide (Moody 1989; Davis 1994). As previously mentioned, crawfish are available live, or boiled ready to eat, but they are also available as picked tail meat for the convenience of consumers and restaurateurs (Romaire and others 2005). Processors peel the crawfish and remove the tail meat, the meat is usually vacuum packed in one-pound packages, frozen, and

sold to restaurants and supermarkets (Davis 1994). Processors that peel tail meat and sell to local consumers can offer crawfish with adhering hepatopancreas or "fat" or tail meat washed of the hepatopancreas. The hepatopancreas is often left on crawfish destined for local markets because of the desired flavor that the hepatopancreas delivers (Horst, 2010). Davis (1994) suggested that crawfish tail meat with adhering hepatopancreas can also be frozen, but typically becomes rancid within 30 days of storage.

There are three phases involved in commercial crawfish processing. There is the storing and cooking of live crawfish (crawfish are generally boiled in clean and unseasoned water if they are destined for the picked tail meat market), picking the meat and removal of waste, and lastly the packaging and storing of the finished product (Moody 1989). When receiving live crawfish, it is important that they are not too hot or too cold because they are very temperature sensitive and susceptible to death if they are not kept at a cool, refrigerated temperature of 40-45°F. If they are properly stored, they can survive several days in the cooler (Moody 1989).

Crawfish are graded based on size using grading equipment that reduces damage and death to the crawfish compared to hand grading. Graders typically feature parallel bars whose spacing increases as the crawfish move along the bars. Smaller sized crawfish pass through and are collected while the larger crawfish continue along the bars until the spacing is such that they pass through the bars or exit the grader (Moody 1989). The larger crawfish are used for the whole, cooked crawfish market or to be sold live while the smaller sized crawfish are used for peeling (Moody 1989; Romaire and others 2005). After crawfish are graded, crawfish destined for the cooked market are thoroughly washed then blanched/boiled in unseasoned water while making sure not to overcook the crawfish, which make them difficult to peel (Moody 1989).

Crawfish are hand-peeled, even on a large commercial level, which is a very time consuming and labor intensive undertaking (Moody 1989; Davis 1994). At large crawfish peeling facilities; there can be between 75 and 100 peelers at the peak of the season. These peelers are generally paid by the weight of tail meat peeled (Moody 1989). After crawfish are peeled they are typically packaged in one-pound polyethylene bags and frozen or packaged on ice (Moody 1989; Davis 1994). The crawfish industry is becoming more and more specialized and is expanding the variety of processed products. Twenty years ago, processors began to focus on value added products such as microwave-ready dishes and precooked/specialty dishes (Davis 1994). The increase in the development of more of these value-added products will allow crawfish to reach a broader consumer base and domestic distribution.

2.2 Crawfish Composition

The composition of crawfish including the proximate, mineral, and fatty acid contents are important basic scientific knowledge for understanding the chemistry and changes that occur during the refrigerated and frozen storage of the whole crawfish. The composition of cooked, farmed, mixed species of edible crawfish tail meat from the USDA Nutrient Database for Standard Reference Release 27 (USDA 2014) and data on boiled crawfish tail meat from mixed species of crawfish by Sidwell (1981) are in Table 1. Neither the Sidwell nor the USDA results indicate whether or not the crawfish analyzed had adhering hepatopancreas. Values are also presented in Table 1 to give an idea of expected values for selected minerals.

Table 1. Composition of Cooked Tail Meat from Mixed Species of Crawfish.

Proximate Composition	**USDA (2014) Boiled Crawfish % of Tail Meat**	**Sidwell (1981) Boiled Crawfish % of Tail Meat**
Moisture	79.37	75.0
Protein	16.77	19.4
Ash	1.2	4.4
Total Lipid (Fat)	1.2	0.8
Sum of Moisture, Protein, Ash and Total Lipid	**98.54**	**99.6**
Minerals	**USDA (2014) Boiled Crawfish ppm of Tail Meat**	***Sidwell (1981) Raw Crawfish ppm of Tail Meat**
Calcium, Ca	510	650 - 2,700
Iron, Fe	11	9 - 373
Magnesium, Mg	330	1,930 - 2,000
Phosphorus, P	2,410	1,010 - 1,920
Sodium, Na	970	1,820
Zinc, Zn	14.8	16.38
Potassium, K	2,380	5,000

* Ranges are given by Sidwell (1981) to indicate the large variation in amount of minerals present.

2.3 Microbiology and Degradation of Crawfish and Crawfish Products

Crawfish, like other fish, are a very fragile and perishable product. Post mortem changes occur rapidly and cause a loss in value even before spoilage due to off odors and flavors (Miget 1991; Zeng and others 2005). Peeled crawfish tails are in high demand and have a solid

consumer market, especially in Louisiana, however, their shelf life can be limited. Peeled tails are perishable, lasting roughly a week under refrigeration at 40°F (4.4°C), and 12-16 days on ice (Lovell 1968). This limited shelf life is due to endogenous proteolytic processes, microbial spoilage and oxidative processes such as the lipid and protein oxidation associated with physiochemical changes and off-flavors (Godber and others 1989; Chen and others 2007).

Ehira and Uchiyama (1987) concluded that a decline of freshness in seafood products occurs long before significant bacterial action occurs. This initial reduction in freshness is due to autolytic reactions by native enzymes in the tissue (Ehira and Uchiyama 1987). This is particularly the case in crawfish which contain a hepatopancreas that has proteolytic enzymes which have been implicated in the development of a mushy texture in processed crawfish, especially undercooked crawfish and crawfish in iced storage (Moertle and others 1985; Godber and others 1989; Kim and others 1996). Crawfish degradation is mainly exhibited in a mushy texture after relatively short periods of iced storage, but deterioration of texture has also been observed in crawfish through extended frozen storage (Godber and others 1986).

Microorganisms are also a major concern in the spoilage of crawfish and are a major focus for the crawfish industry (Cox and Lovell 1973; Zeng and others 2005). There is a focus on bacteria that have public health significance. Crawfish, farmed or wild caught, are apt to come in contact with harmful organisms because of their habitats receiving runoff (Miget 1991). Grodner and Novak (1974) expected total coliforms, *E. coli*, and fecal steptococci to be observed in crawfish and crawfish products because these microorganisms were commonly isolated in waters where crawfish are harvested. Another microbiological concern regarding crawfish is that they are hand-peeled which may promote contamination by pathogenic and spoilage microorganism if proper sanitation practices are not followed (Lovell and Barkate 1969).

An aerobic plate count (APC) is a recommended technique to determine the quality of fish products from a microbiological standpoint (ICMSF 1986). Most seafood products have counts in the range of $10^{2} - 10^{5}$ organisms per gram when they are harvested. There are exceptions such as some tropical shrimp, mollusks, and freshwater fish that can have higher initial counts. An increase in APC to levels exceeding 10^{6} per gram is generally a sign of a long period of time at refrigerated temperatures or temperature abuse before freezing (ICMSF 1986). APC is a good measure of general quality and can also indicate how well a product has been thermally processed and handled.

Aerobic plate count (APC) and *E.col/*coliform limits have been suggested by the International Commission on Microbiological Specifications for Foods (ICMSF 1986). The limits are appropriate for both refrigerated and frozen cooked crawfish. For APC, a class-3 plan with five samples taken should not have more than three samples between log 5.7 colony forming units per gram (CFU/g) and log 7 CFU/g. Any sample having an APC count exceeding log7 CFU/g should result in the product being discarded. For *E.coli/*colifoms using a class-3 plan with five samples taken, no more than 3 samples can be between 11 and 500 CFU/g. Any sample exceeding 500 CFU/g should result in all samples being discarded. For this study, these limits provided the determining factor for acceptability of refrigerated or frozen crawfish. For this study, if the average CFU/g for APC or *E.coli*/coliform exceeded the upper limit (log7 CFU/g), the sample was considered unfit.

CHAPTER 3: MATERIALS AND METHODS

3.1 Procurement

Live freshwater crawfish consisting of both red swamp crawfish (*Procambarus clarkii*), and white river crawfish (*Procambarus zonangulus*) were obtained March 19th, 2013 from Louisiana State University Agricultural Center Aquaculture Research Station in Baton Rouge, LA and from Tony's Seafood Market and Deli of Baton Rouge, LA. The live crawfish were transported to the Food Science building in Baton Rouge, LA in mesh sacks varying in weight from 26 to 40 pounds and stored two days at 4°C until processed.

3.2 Cooking

3.2.1 Preparation

Prior to cooking the crawfish were removed from the mesh sacks and placed in 1.5m x 0.6m x 0.6m metal lugs. The crawfish from the different locations were mixed and washed with municipal tap water to remove any mud and debris. Dead crawfish were separated from the live crawfish and discarded. The crawfish were then kept moist by regularly spraying them with municipal tap water until they were processed.

Figure 2. Crawfish in Metal Lugs.

3.2.2 Boiling

Two lots of 28.8 kg of crawfish destined for boiling were removed from the metal lugs and placed in a stainless steel basket. Thermocouple wires were inserted into two crawfish tails prior to cooking to measure internal temperature during the cook process. Temperatures were obtained with an Omega OM-DAQPRO-5300 T-type thermocouple and data logger (Omega Engineering Inc., Stamford, CT). The crawfish were placed in approximately 75.7 L of unseasoned tap water that had been brought to a boil at atmospheric pressure in a 150 L jacketed steam kettle (B.H. Hubbert & Son, Inc., Baltimore, MD). University-generated 206.85 cmHg (40psi) food-grade steam was the source of heat. The temperature of the water in the kettle was measured at periodic (~30 second) intervals during the cook process using a Comark C28 K-type thermocouple (Comark Instruments, Norfolk, England). Crawfish were lowered in to the steam kettle after the water had been brought to a boil. The crawfish were completely submerged in the water and continuously stirred with a plastic paddle to ensure that the crawfish were evenly heated. It took seven minutes for the water to return to a boil. The crawfish were then boiled for exactly three minutes. The cook time of ten minutes was suggested by D&T Crawfish Company in Abbeville, Louisiana which satisfied the adequate cook time (Marshall and others 1987) of seven minutes or more that would ensure the deactivation of proteolytic enzymes and prevent mushiness of the tail meat that is caused by enzymatic hydrolysis.

At the end of the three-minute boil, steam was immediately turned off and several crawfish were retrieved to measure internal temperature using the Comark C28 K-type thermocouple. The thermocouple was inserted into the thickest part of the tail to record the most accurate internal temperature. The internal temperature of the crawfish from the boiling trials ranged from 86.7-91.1°C (188-196°F). Once the steam was turned off, and internal temperatures

measured, the basket containing the crawfish was raised out of the kettle and then submerged in a large sanitized plastic lug (1.2m x 1.2m x 0.9m) containing ice-water to rapidly cool the crawfish and prevent continued cooking. The crawfish were allowed to cool in the ice water with periodic stirring. After five minutes of cooling, the crawfish were hoisted out of the chilled water; temperatures were measured using the Comark C28 K-type thermocouple again by inserting the thermocouple into the thickest part of the crawfish tail. The crawfish were poured from the basket into plastic lined, waxed fish boxes (0.6m x 0.3m x 0.3m) obtained from Tony's Seafood Market and Deli of Baton Rouge, LA. The crawfish were then placed in a cooler at 5°C to further cool for 4 hours prior to being separated into treatments of refrigerated storage and frozen storage at -18°C°.

All of the boiled crawfish were mixed together after they had been refrigerated for four hours after cooking. They were again mixed together to make the lot as homogenous as possible. This was done by combining all of the crawfish from the boiled treatments and carefully mixing in clean plastic lined fish boxes by hand so as not to break any claws off.

Figure 3. Crawfish Being Boiled in a Jacketed Steam Kettle.

3.2.3 Steaming

A commercial batch vegetable blancher was used to steam the crawfish. Because of the limited capacity of the blancher, eight trials of approximately 7.3 kg of live crawfish were conducted. This resulted in the total amount of crawfish cooked to be nearly the same for the two cooking methods. Live crawfish (~7.3kg) were placed in stainless steel trays and inserted into the chamber of the blancher and the lid was closed. Omega OM-DAQPRO-5300 Type-T thermocouples connected to a data logger (Omega Engineering Inc., Stamford, CT) were placed in the tails of two crawfish per trial. Initial temperatures (uncooked) and final temperature of the crawfish during the cook process were recorded. The crawfish were steamed in the blancher using food-grade steam at 206.86 cmHg (40psi) to approximately the same internal temperature (90-92.2°C) reached during the boiling process. The crawfish were immediately removed from their trays and placed in an ice bath similar to that used after the boiling process and allowed to cool for five minutes with periodic stirring. Temperatures were measured after cooling and then the crawfish were placed in plastic lugs (0.6m x 0.3m x 0.3m) and stored in a cooler at 5°C to further cool for four hours prior to being separated for the refrigerated storage at 4°C and frozen storage at -18°C treatments.

Crawfish destined for frozen storage were placed into plastic lined, waxed fish boxes (0.6m x 0.3m x 0.3m) and filled approximately half way full. The plastic was wrapped around the crawfish to expel as much air as possible and then the lid of the box was placed on the box. The boxes were then placed in the -18°C freezer and stored. At monthly intervals, roughly four kilograms of crawfish were removed from the waxed fish boxes and placed in foil trays, covered, and thawed under refrigeration (4°C) over night.

Figure 4. A Picture of the Vegetable Blancher used to Steam the Live Crawfish

3.3 Gelatin Test

In this study, a gelatin test (Marshall and others 1987) was used to determine if the cook time for crawfish was sufficient enough to deactivate proteolytic enzymes. The test was conducted on steamed, boiled, and raw crawfish samples. Following the 4-hour cooling period at 5°C, five g of hepatopancreas from each cooked treatment and five grams of raw crawfish hepatopancreas were obtained by peeling the tails of 5-10 crawfish of each treatment. The hepatopancreas was then minced using a metal spatula and set aside at 5°C until used. Then, in triplicate for each treatment, 0.2 g of mince was placed into labeled 22 mm Pyrex® tubes (Corning Corporation, Tewksbury, MA). Concurrently, blanks in triplicate, which contained no hepatopancreas, were prepared. Five ml of cooled 12% Knox® gelatin (Kraft Foods Group, Inc., Northfield, IL) in water were then added to each tube and the contents homogenized using a Vortex-Genie® 2 mixer (Scientific Industries Inc., Bohemia, NY).

The samples were then allowed to incubate for 1h at room temperature followed by holding at 3°C for 23 h. After the 23 hour refrigerated storage, the samples were removed and analyzed subjectively to determine the presence of a firm gel. A loose gel or no gel formation at

all would suggest that proteolytic enzymes present in the hepatopancreas had not been deactivated whereas firm gels would suggest the enzymes had been deactivated by an adequate cooking process.

3.4 Microbiological Analysis

Microbiological analyses conducted on the crawfish included the quantification of the aerobic plate count (APC) and *E.coli*/coliforms of refrigerated samples on days 0, 1, 3, 5, 7, 9, and 11 and on frozen samples at months 0, 1, 2, 3, 4, 5, and 6. APC and *E.coli*/coliform tests were also conducted on the water from the ice water baths in which the boiled and steamed crawfish were submerged after to stop the cook process. Analyses were also conducted on raw crawfish tail meat for comparison to cooked tail meat.

Crawfish tail meat with adhering hepatopancreas was removed by hand peeling and placed in 17.78cm x 19.05cm Qwik Seal® reclosable storage bags (Reynolds®, Lake Forest, Illinois). The peeled tail meat was immediately transported on ice to the Food Microbiology Lab in the Agricultural Chemistry building on LSU's campus. Twenty-five gram samples (in duplicate for each treatment) were prepared and placed in 1.56kg Whirl-pak® bags (Nasco, Fort Atkinson, WI) along with 25.0 mL of phosphate buffered saline (PBS), which was composed of 0.24% sodium phosphate monobasic (Sigma-Aldrich Corporation, St. Louis, MO), 0.28% sodium phosphate dibasic (Sigma-Aldrich Corporation, St. Louis, MO), and 0.85% sodium chloride (Amresco LLC, Solon, OH). The 25 g of tail meat and 25 mL of PBS were homogenized for 60 seconds using EasyMix blender (AES Chemumex, Bruz Cedex, France).

From each treatment, serial dilutions were prepared and samples plated on both 3M™ *E.coli*/Coliform and Aerobic Count Petrifilms™ (3M Company, St. Paul, MN). The petrifilms were then incubated at 35° ± 1°C. *E.coli* and coliforms were determined and recorded after 24 ±

3 hours and aerobic plate count (APC) was counted and recorded after 48 ± 3 h (AOAC Official Methods 991.14 and 998.08 for E.coli/coliforms and AOAC Official Methods 990.12 for aerobic plate count, 2005).

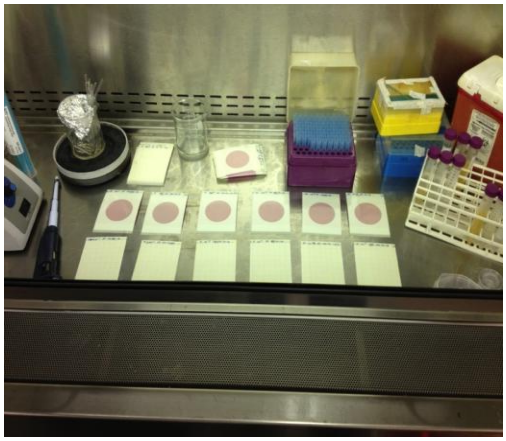


Figure 5. Hood and Petrifilms used for Coliform (Above/Red) and Aerobic (Below/Yellow) Counts.

3.5 Color Analysis

Using a calibrated Minolta CM-508d Spectrophotometer (Konica Minolta Sensing Americas Inc., Ramsery, NJ), Hunter color scale values (L*, a*, and b*) were measured on ten randomly selected tails with hepatopancreas at the widest point at the back of the peeled tails for both boiled and steamed crawfish and recorded. L* represents the degree of lightness, a* represents degree of redness, and b* represents the degree of yellowness. Measurements on refrigerated samples were conducted on days 1, 3, 5, 7, 9, and 11 of refrigeration (4°C) and on frozen samples on months 1, 2, 3, 4, 5, and 6 of frozen storage (-18°C).

3.6 Texture Analysis

Texture analysis was conducted using a TA-XT Plus Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY) with a 5-blade Kramer shear attachment and a 30 kg

load cell. Texture of peeled tails with adhering hepatopancreas from both boiled and steamed treatments was measured on days 0, 1, 3, 5, 7, 9, and 11 for refrigerated samples and after 0, 1, 2, 3, 4, 5, and 6 months of frozen storage. Peak shear force (kg) and work of shearing (kg*m/s) were measured on 100 grams of peeled tail meat from each treatment in triplicate. Temperature at the time of analysis was at 23 ± 1 °C as measured with a Taylor 9878E digital thermometer (Taylor Precision Product, Oak Brook, IL). One hundred grams of sample was placed randomly in the Kramer cell, which filled the cell to roughly 40-50% capacity. The blade was set at 45 mm (~10mm above the sample) and the blade speed was set at 3mm per second. Following the texture determination, the samples were fully homogenized using an Oster® Osterizer 14 speed all metal drive blender (Jarden Consumer Solutions, Providence, RI) and used for subsequent proximate and TBAR analyses.

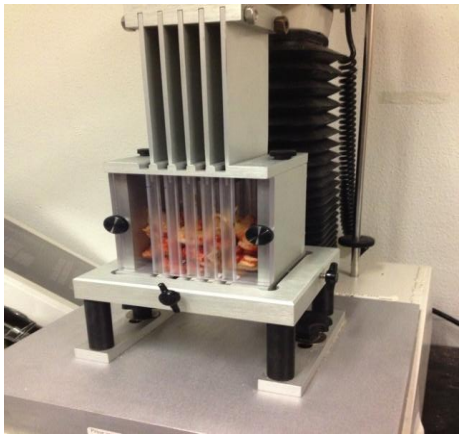


Figure 6. Texture Analyzer with a 5-blade Kramer Attachment used for Simultaneous Measurement of Shear Force and Work of Shearing.

3.7 Proximate Analysis

Proximate analysis (moisture, ash, protein, and fat) was conducted on refrigerated crawfish with adhering hepatopancreas on days 0, 1, 3, 5, 7, 9, and 11 and on frozen crawfish with adhering hepatopancreas at months 0, 1, 2, 3, 4, 5, and 6 for both boiled and steamed samples.

3.7.1 Moisture Analysis

Moisture analysis was conducted in triplicate for each cooking treatment. Three grams of homogenized tail meat were placed in ceramic crucibles, weighed, and then placed in a Model 20 GC Lab Oven (Quincy Lab Inc., Chicago, IL) at 100°C for 24 hours. Samples were removed from the oven and placed in a desiccator to cool before weighing. The percent moisture of tail meat with adhering hepatopancreas was determined by the calculation: % Moisture = [(Wet Weight – Dry Weight)/Wet Weight]×100.

3.7.2 Ash Analysis

Ash measurements were conducted on the samples after moisture determination. The samples (in triplicate) were placed in a Type 6000 Furnace (Thermolyne Inc., Dubuque, IA) and heated to 550°C for 18-24 hours. Samples were removed from the furnace and placed in a desiccator to cool before weighing. The percent ash was determined using the calculation: %Ash = (Weight of sample and crucible after ashing – Tare Weight of Crucible)/(Weight of Dry Sample and Crucible after Oven Drying – Tare weight of Crucible) × 100.

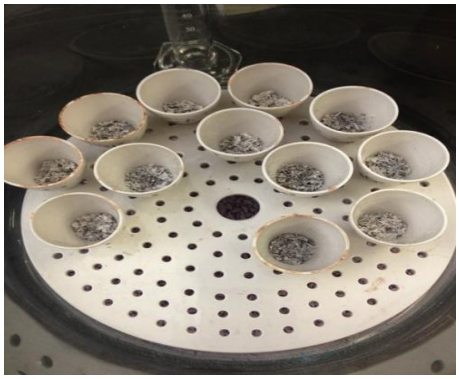


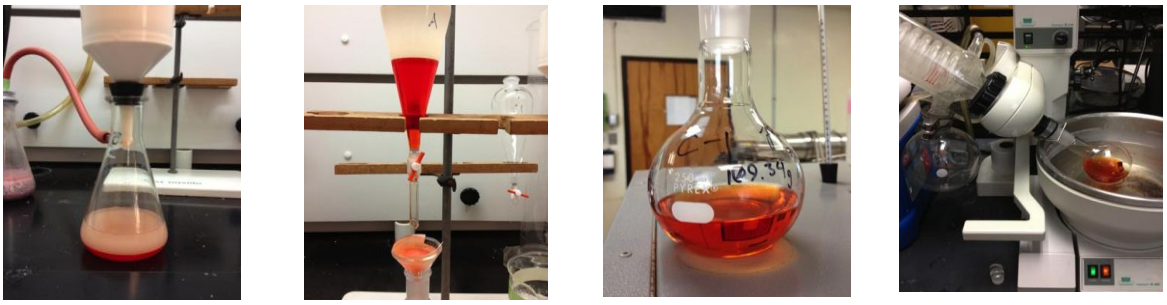
Figure 7. Samples in Ceramic Crucibles after Moisture Analysis and Subsequent Ashing.

3.7.3 Lipid Analysis

Lipid extraction was performed according to a modified method of Bligh and Dyer (1959) described by Woyewoda and others (1986). Total lipid quantification was performed in duplicate for each treatment. Following lipid quantification on a wet weight basis, fatty acid analysis was conducted on the lipid. The process was begun by homogenizing peeled crawfish tail meat with adhering hepatopancreas in a glass jar on a Oster® Osterizer 14 speed all metal drive blender with a rosin blade (Jarden Consumer Solutions, Providence, RI). A measured amount of homogenized tail meat (approximately 50.0 grams) was added to a Waring® model 51BL31 commercial blender (Waring®, Stanford, CT) along with 100.0 mL of HPLC grade anhydrous methyl alcohol (Avantor™ Performance Materials Inc., Center Valley, PA) and 50.0 mL of HPLC grade Chloroform (Mallinckrodt Baker Inc., Phillipsburg, NJ). The homogenized tail meat along with the methanol and chloroform were blended for exactly two minutes. An additional 50.0 mL of chloroform was added to the blender and the mixture was blended for an additional 30.0 seconds. The mixture was then filtered through a Buchner funnel containing a Whatman™ #1 filter paper (GE Healthcare UK Limited, Buckinghamshire, UK). Aspiration was used to expedite the filtering process. The filtered product contained lipid, chloroform, and methanol. Fifty mL of distilled water was added and the mixture was then stirred vigorously. The mixture was transferred to a 250-mL separatory funnel and then allowed to rest at 5°C over night.

The following day, the chloroform-lipid layer was then filtered from the separatory funnel through a glass funnel containing a Whatman™ #4 filter paper inside a Whatman™ #1 filter paper (GE Healthcare UK Limited, Buckinghamshire, UK) that was filled with ACS grade anhydrous sodium sulfate (ThermoFisher Scientific, Waltham, MA) into a pre-weighed 250 ml

round bottom boiling flask (Corning Corporation, Tewksbury, MA). The chloroform was then removed from the round bottom flask using a Buchi Rotovapor R114 rotoevaporator (Buchi Corporation, New Castle, DE). To ensure that all solvent was removed and only crude lipid remained, ultra high pure (UHP) nitrogen (Air Liquide Corporation, Paris, France) was sprayed into the flask for 10-15 minutes or until no odor or appearance of solvent remained. The crude lipid remaining was weighed and the percent fat was determined by the calculation: % Fat = [(Weight of Flask Containing Lipid – Weight of Empty Flask)/ Weight of Sample Used] × 100.



A. **B.** **C.** **D.**

Figure 8. Steps Involved in the Solvent Extraction of Fat from Crawfish Tissue and Removal of Solvents to Yield Crude Lipid for Quantification, Derivation, and Fatty Acid Analysis. A. Filtering with Buchner funnel, leaving chloroform (lipid) and methanol (aqueous) layers. B. Filtering lipid layer through anhydrous sodium sulfate. C. Lipid in chloroform. D. Removal of the chloroform to yield crude lipid.

3.7.4 Protein Analysis

Protein analysis was conducted on homogenized tail meat with adhering hepatopancreas from each cooking method. The samples were dried in the same manner as samples dried for moisture analysis. The dried samples were then blended to a fine powder using a mortar and pestle and Custom Grind™ coffee grinder (Hamilton Beach Brands Inc., South Pines, NC). Approximately 1.5 grams of finely ground sample were placed in 15 ml clear, screw top vials (Supelco®, Bellefonte, PA) and transported to the Soil Testing & Plant Analysis Laboratory on the LSU campus for combustion and total nitrogen determination. Total nitrogen determination

was conducted on 0.25 gram samples in triplicate using a LECO® TruSpec Micro CHNS analyzer (LECO Corporation, St. Joseph, MI). The results were reported as percent total nitrogen in the sample. The total percent protein was determined by multiplying the percent total nitrogen by the appropriate conversion factor of 6.25 and converting from a dry weight basis to a wet weight basis.

3.8 pH Analysis

pH of crawfish tail meat with hepatopancreas was measured in triplicate for each treatment using a Milwaukee SMS115 pH meter (Milwaukee Instruments Inc., Rocky Mount, NC) that had been calibrated at pH values of 4, 7, and 10. Ten grams of homogenized crawfish tail meat were blended with 90 mL of distilled/de-ionized water for one minute using a Waring® model 51BL31 commercial blender (Waring, Stanford, CT). The samples were then transferred to 400 mL Pyrex® beakers (Corning Corporation, Tewksbury, MA) and the pH was measured and recorded.

3.9 TBARS Analysis

Thiobarbituric acid reactive substances (TBARS) analyses were conducted on boiled and steamed crawfish tail meat refrigerated for 0, 1, 3, 5, 7, 9 and 11 days and on frozen samples at months 0, 1, 2, 3, 4, 5, and 6 using a modified method of Vyncke (1970) by Lemon (1975). Solutions were prepared the day prior to analysis. The extraction solution consisted of 7.5% trichloroacetic acid (TCA) (ThermoFisher Scientific, Waltham, MA), 0.1% propyl gallate (Sigma-Aldrich Corporation, St. Louis, MO), and 0.1% ethylene diaminetetraacetic Acid (EDTA) (Sigma-Aldrich Corporation, St. Louis, MO) in deionized water. The thiobarbituric acid (TBA) solution consisted of 2.883 g/L (0.02M) of TBA (Sigma-Aldrich Corporation, St. Louis, MO) in deionized water. The standard solution for standard curve determination was prepared by

dissolving 0.22 grams of 1,1,3,3-Tetraethoxypropane (TEP) (Sigma-Aldrich Corporation, St. Louis, MO) in one L of water. The working solution to actually obtain the standard curve was formed by diluting the standard solution 100 fold.

Fifteen grams of tissue from each treatment (in triplicate) were blended with 30 ml of extraction solution for 30 seconds using a Waring® blender. The samples were then filtered through a Whatman #1 filter paper into a 100 ml Pyrex® beaker. Five mL of the clear filtrate in the beaker was added to five ml of TBA reagent in Pyrex® (120 x 10 mm) test tubes with screw caps. The test tubes were then heated in boiling water in 1000 ml Pyrex® beakers on a Corning PC-420D hot plate (Corning Corporation, Tewksbury, MA). Test tubes containing only five mL of water and five ml of TBA reagent were added to beakers as blanks. After boiling for exactly 40 minutes, the tubes were removed from the beaker and cooled in running tap water.

Using a transfer pipette, each sample was transferred to a cuvette and their optical density was measured at 530nm against the blanks of water and TBA reagent using a Thermo Spectronic Genesys™ 2 spectrophotometer (ThermoFisher Scientific, Waltham, MA). The TBA values were calculated from the standard curve obtained from the TEP working solution and the values were reported in mg malonaldehyde (MDA) equivalent/100 grams of tissue.

Figure 9. Test Tube Holder Containing the Standard Curve (*1-*6) Solutions and Test Samples Behind (S-1, etc.) for TBARS Analysis.

3.10 Mineral Analysis

Mineral analyses were conducted on the ashed samples remaining from the proximate analysis of boiled and steamed crawfish tail meat that had been refrigerated for days 0,1,3,5,7,9, and 11 and frozen samples on months 1,2,3,4,5, and 6. Ten mL of 10% nitric acid solution in distilled water (Avantor™ Performance Materials Inc., Center Valley, PA) were added to each crucible for ten minutes to solubilize the ash. The solubilized ash samples were drawn into a sterile 10 mL Luer-Lok™ tip syringe (Becton Dickinson & Company, Franklin Lakes, NJ). A 0.2µm, 25-mm surfactant free cellulose acetate membrane, acrylic housing, Nalgene™ syringe filter was placed on the syringe (ThermoFisher Scientific, Waltham, MA) and the sample was filtered into labeled 15 ml clear, screw top vials (Supelco®, Bellefonte, PA). Two of the three samples were then transported to the LSU AgCenter's W.A. Callegari Environmental Center Central Research Station in Baton Rouge, Louisiana for analysis in duplicate via inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Varian Vista-MPX CCD Simultaneous ICP-OES unit (Varian Medical Systems Inc., Palo Alto, CA). The results for minerals present in the samples were reported in parts per million (ppm) of crawfish tail meat with adhering hepatopancreas on a wet weight basis.

3.11 Fatty Acid Analysis

Fatty acid profiles were obtained from samples of lipid collected during proximate analysis (3.6.3). In duplicate, 1.3-2.0 grams of crude lipid was solubilized in exactly ten ml of HPLC grade Hexane (Honeywell, Morristown, NJ) and transferred to a 15 mL clear, screw top vial (Supelco®, Bellefonte, PA). Samples were then placed in the freezer (-18°C) until they were transported to the LSU AgCenter's W.A. Callegari Evnvironmental Center Central Research Station in Baton Rouge, Louisiana for analysis. Fatty acid analysis was conducted using a Varian

450 gas chromatograph with a Varian 240 ion trap mass spectrometer (Varian Inc., Palo Alto, CA) with SP2560 75 meter, 0.18 mm diameter, 0.14 μm film thickness, silica capillary column (Supelco®, Bellefonte, PA) with hydrogen as the carrier gas at a flow rate of 40 cm per second.

3.12 Statistical analysis

Results are expressed as least squares means (LS-Means) ± standard deviation. The experimental design used was a 2x7 factorial design for both refrigerated and frozen studies. Statistical analysis was performed using analysis of variance (ANOVA). Separation of means and difference between control and treatments were determined by the generalized linear model (GLM) procedure with a T comparison for least squares means (SAS, version 9.3). Statistical significance was set at P-value < 0.05.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Cooking, Cooling, and Gelatin Test

Crawfish were cooked then cooled as rapidly as possible. Submerging the cooked crawfish in the ice bath immediately after cooking was done to retard the cook process and to cool the crawfish within recommended guidelines (FDA 2011) and to promote product quality. Controlling the temperature of the product and keeping it as low as possible 1) slows the rate of spoilage reactions that include bacterial and autolytic enzyme activity and 2) reduces the rate at which bacteria multiply (Ronsivalli and Charm 1975).

4.1.1 Boiling

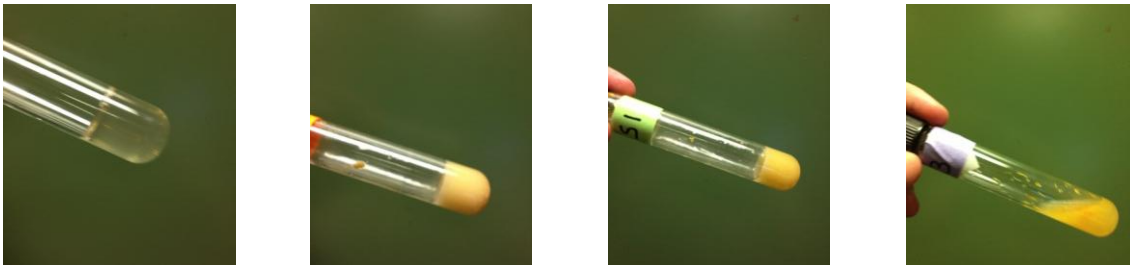
An average internal temperature of 87.6° C (189.6°F) was obtained (5 crawfish tempted per each of 2 replications) for the crawfish receiving the boiling treatments. This average internal temperature provides an immediate death of the target pathogen *Listeria monocytogenes* which exhibits a 100% mortality at 85°C (185°F) for 0.02 minutes (FDA 2011). This temperature was met or exceeded for this project in order to mimic industry practices. Cooked crawfish are a ready-to-eat (RTE) product and there is a zero tolerance for *L. monocytogenes* (FDA 2011). After the cook process, the crawfish were submerged in an ice bath for five minutes and the internal temperatures recorded for five crawfish chosen randomly. The average chilled temperature of the crawfish after the ice bath for the two trials was 30.11°C (86.2°F). After four hours of cooling in refrigeration, the temperature of all the crawfish was reduced to an average of 3.5°C (39.8°F).

4.1.2 Steaming

There were a total of eight replications of steaming. The average internal temperature of the cooked tails was 86.9°C (188.4°F). The crawfish were steamed until they reached an internal temperature essentially the same as that of the crawfish that had been boiled. The internal temperature of the steamed crawfish was very close to the average temperature of the boiled samples, and is also a sufficient temperature to have a 100% lethality of *Listeria monocytogenes* (FDA 2011). After the ice bath, the average internal temperature of the steamed samples was 29.6°C (85.28°F). The slightly lower internal temperature of the boiled samples might be due to the smaller batch size of ~7.26 kg (16 lbs.) versus the boiled batch sizes which were ~ 27.22 kg (60lbs.). Once the steamed samples had been boxed and allowed to cool under refrigeration for four hours, the average internal temperature was 3.5° (39.8°F), the same as that of the boiled samples.

4.1.3 Gelatin Test

Figure 10 depicts results of the gelatin test. Sample A is the control sample that contained only gelatin and no hepatopancreas. A firm gel formed after the refrigerated period, as indicated by a lack of flow of the material. Boiled (B) and steamed (C) crawfish samples contained cooked hepatopancreas. Both B and C formed stable, firm gels after cooking. According to Marshall and others (1987), this indicates that the cook time was sufficient to deactivate the proteolytic enzymes in the hepatopancreas; otherwise a firm gel would not have been able to form. Sample D contains uncooked hepatopancreas with active proteolytic enzymes which prevent the formation of a firm, stable gel to form.



A. **B.** **C.** **D.**

Figure 10. Gelatin Test Results for Control (A), Boiled and Steamed Hepatopancreas (B & C), and Uncooked, Raw Hepatopancreas (D).

It is important to render these enzymes inactive to prevent mushiness of the meat from occurring. The heat-labile proteolytic enzymes present in the hepatopancreas of crawfish promote the development of mushiness in the tail meat (Kim and others 1996). Rowland and others (1982) proposed that these proteolytic enzymes diffuse from the digestive tract into the tail meat from either the hepatopancreas, which is located at the anterior part of the tail, or from the intestine along the length of the tail (Rowland and others 1982).

This is particularly relevant to this study because the crawfish were cooked and refrigerated whole, so the hepatopancreas would be in contact with the tail meat for the entirety of the storage. If the crawfish were cooked, cooled, and then peeled/deveined immediately, the choice of cook time and temperature would not have been partially dependent upon deactivating the proteolytic enzymes.

4.2 Yield Results

The yields obtained for both the boiled and the steamed crawfish through refrigerated and frozen storage are shown in Table 2. It was expected that the edible yield would decrease during storage, especially frozen storage and thawing due to drip loss. This is a familiar issue in frozen fish products where physical damage is related to the rate of freezing. Slow freezing causes large ice crystal formation that damages cell walls and promotes water loss upon thawing (Sikorski and others 1976). For that reason, it was important to cool the crawfish to as low a temperature as practical prior to actual freezing to reduce the freezing time.

Since crawfish are generally hand peeled, there can be variations in the yield obtained from peeling depending on the technique and experience of the peeler. The average abdominal (tail) meat yield for cooked crawfish is about 15% of the live weight of the crawfish (Romaire and others 2005). The yield also depends on the sexual maturity of the crawfish, with immature crawfish having a higher yield because they have smaller claws and thinner shells (Romaire and others 2005). This is typical of crawfish early in the season, when yields can be as high as 22 to 23%. Later in the season, when the crawfish have matured and have larger claws and thicker shells, the yield can be as low as 10 to 11% (Romaire and others 2005).

The yields obtained in this study average around 18%. This was higher than expected because these crawfish were harvested later in the season and their shells were quite thick. A reason why the yields may have been higher than expected is that the crawfish were very carefully peeled. Also, the hepatopancreas was left attached as well as the vein/intestine that runs along the dorsal side of the abdomen/tail. Typically this is removed during commercial peeling but was left on in this research because the goal was to mimic how people typically consume boiled crawfish in Louisiana.

Table 2. Percent (%) Edible Yield of Peeled Crawfish Tail Meat Refrigerated for Days 0,1,3,5,7,9, 11 and Frozen for and Thawed at Months 0,1,2,3,4,5, and 6 of Frozen Storage. Yield based on % of Whole, Cooked, and Thawed (Frozen Samples) Crawfish.

Refrigerated	**Day 0**	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day9**	**Day 11**	**Avg. % Yield**
Boiled	19.14	18.41	20.16	18.09	18.02	19.46	17.43	18.67 ± 0.95
Steamed	18.58	18.63	18.60	17.84	17.63	19.19	15.80	18.03 ± 1.11
Frozen	**Mo. 0***	**Mo. 1**	**Mo. 2**	**Mo. 3**	**Mo. 4**	**Mo. 5**	**Mo. 6**	**Avg. % Yield**
Boiled	19.14	17.69	17.36	19.16	17.28	18.13	17.52	18.04 ± 0.81
Steamed	18.58	17.08	17.98	18.71	16.84	18.92	19.45	18.22 ± 0.96

***** At Month 0, samples yield was determined prior to freezing.

4.3 Proximate Analysis Results

The proximate analyses LS-means for the refrigerated and frozen crawfish tail meat with the hepatopancreas attached are in Table 3. The LS-means are separated based on cooking method (boiled/steamed) as well as by storage type (refrigerated/frozen). The proximate analyses from the USDA (2014) and Sidwell (1981) are also shown for comparison. The values from the USDA are for cooked crawfish tails. Sidwell (1981) presented results for both cooked and raw tail meat. It was not specified whether or not the tail meat samples included hepatopancreas in the USDA (2014) or in the Sidwell (1981) studies. The USDA also did not state if the tail meat underwent any type of storage before analysis. A more in-depth breakdown of the proximate analyses is presented later.

Table 3. Means for % Moisture, Ash, Protein, and Fat of Crawfish Tail Meat During Refrigerated (3°C) and Frozen (-18°C) Storage. Proximate Values from USDA (2014) and Sidwell (1981) Are Listed for Comparison.

	Boiled	**Steamed**	**USDA (2014) Boiled**	**Sidwell (1981) Boiled**	**Sidwell (1981) Raw Range**
% Moisture (3°C)	79.29	79.40	79.37	75.0	(72.1 - 83.4)
% Moisture (-18°C)	77.91	77.86			
% Ash (3°C)	1.26	1.31	1.2	1.5	(0.7 - 3.6)
% Ash (-18°C)	1.22	1.32			
% Protein (3°C)	15.89	15.30	16.77	16.3	(11.9 - 24.1)
% Protein (-18°C)	16.69	16.30			
% Fat (3°C)	2.73	2.96	1.2	0.8	(0.5 - 2.5)
% Fat (-18°C)	3.00	3.22			
Total (3°C/18°C)	**99.17/98.82**	**98.97/98.70**	**97.34**	**98.9**	

4.3.1 Moisture Results

The moisture content of the peeled crawfish tail meat with adhering hepatopancreas measured through refrigerated and frozen storage from both boiled and steamed treatments did not vary greatly. There was little change in the moisture content for the boiled samples through refrigerated storage (days 0-11). As shown in Table 4, the moisture contents on days 0, 1, 3, and 9 were not significantly different from each other and on days 5, 7, and 11 the moisture contents were not significantly different from each other but days 5, 7, and 11 were statistically higher than days 0, 1, 3, and 9. The moisture content for the steamed samples varied a bit more than the boiled samples throughout the 11-day storage. The average moisture contents for the boiled and steamed samples during refrigerated storage for eleven days were 79.29% and 79.40%

respectively. There was no significant difference in moisture between the boiled and steamed samples when compared for the same day of refrigerated storage. This indicates that there is more variation due to the length of storage during refrigeration than to the method of cooking. The differences in moisture could be from natural variation. The large range of moistures (72.1 - 83.4%) observed by Sidwell (1981) is greater than the range of moisture (78.22 to 80.38%) observed over the 11 days of refrigerated storage.

Table 4. Percent (%) Moisture of Peeled Crawfish Tail Meat Under Refrigeration (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	78.23 ± 0.08^{D}	78.77 ± 0.12^{CD}	78.93 ± 0.05^{CD}	80.33 ± 2.04^{AB}	80.19 ± 0.08^{AB}	78.46 ± 0.10^{D}	80.11 ± 0.14^{AB}
Steamed	79.00 ± 0.17^{CD}	79.30 ± 0.02^{ABCD}	78.22 ± 0.05^{D}	79.84 ± 0.36^{ABC}	79.77 ± 0.02^{ABC}	79.27 ± 0.15^{BCD}	80.38 ± 0.11^{A}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

A greater difference in moisture content with cooking treatments was observed between frozen samples (which were thawed prior to moisture determination) than was noted for samples in refrigerated storage. Ice crystal formation, protein denaturation, and increases in salt concentration during frozen storage could have decreased the moisture content of the thawed product (Shenouda 1980; Jittinandana and others 2003). It was thought that ice crystal formation may have been a factor because slow freezing rates cause large ice crystal formation, which would result in cellular disturbance and rupturing (Lampila and others 1985). This would lead to water loss or drip-loss from the crawfish upon thawing. The freezing rate for the crawfish in this study was not measured.

The moisture results do indicate a drop in the percent moisture from the beginning of the study compared to the last sampling six months later, but there was little variation throughout the six months of frozen storage. The average moisture content during frozen storage was 77.91% for boiled and 77.89% for steamed There was a significant difference between the boiled and steamed samples at all time intervals except for months four and five. The largest difference between boiled and steamed samples at any point during frozen storage was 1.24% at month 6, which may influence yields and thus profitability of commercial enterprises.

Table 5. Percent (%) Moisture of Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	78.23 ± 0.08^{BC}	77.05 ± 0.13^{J}	78.36 ± 0.04^{BC}	78.21 ± 0.07^{CD}	77.90 ± 0.06^{EFG}	77.65 ± 0.17^{H}	77.95 ± 0.10^{EF}
Steamed	79.00 ± 0.17^{A}	77.72 ± 0.06^{GH}	77.34 ± 0.14^{I}	78.42 ± 0.11^{B}	78.02 ± 0.26^{DE}	77.79 ± 0.12^{FGH}	76.71 ± 0.04^{K}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

It can be visualized from Figure 11 that there are only minor differences between the two cooking methods. The moisture contents for refrigerated samples are comparable with recorded moisture contents from the USDA (2014) and Sidwell (1981) (Table 3). The USDA and Sidwell did not provide any results on frozen crawfish tails. Results from a study by (Nadarajah and Others, 2013) on the composition of cooked, minced meat from undersized crawfish indicated a moisture content of 80.4% which is also reasonably close to the values obtained in this study. These results from the cited references are in agreement with those of the present study which suggests that there is no meaningful difference, in a commercial sense, between boiling and steaming on the moisture content of crawfish tail meat through refrigerated and frozen storage.

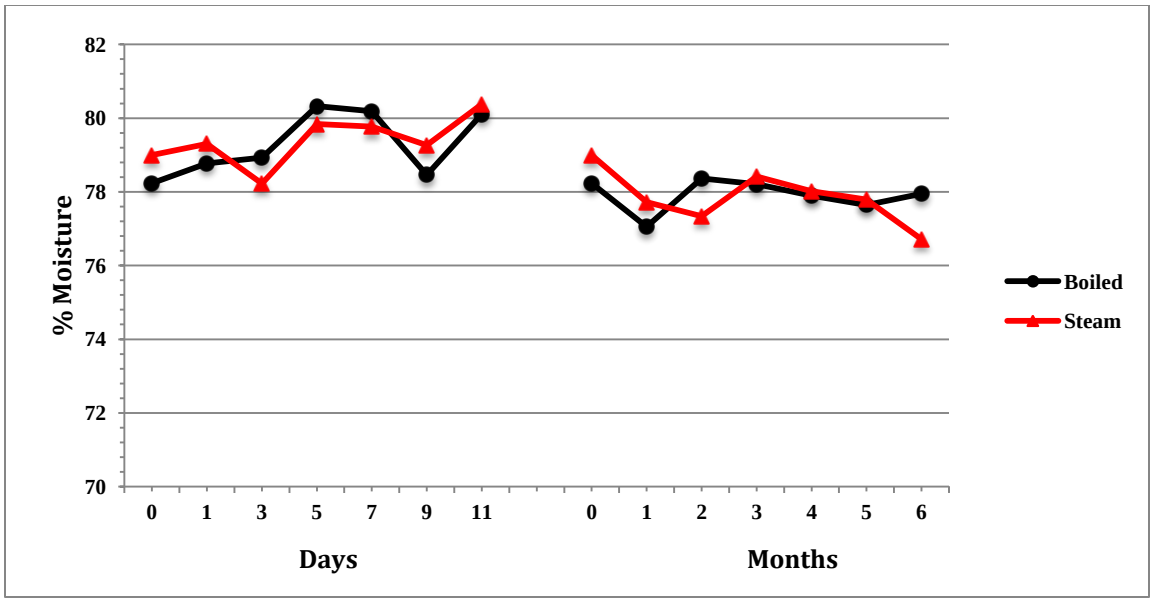


Figure 11. Percent Moisture of Boiled and Steamed Crawfish Tail Meat During Refrigerated (3°C) Days 0, 1, 3, 5, 7, 9, 11 and Frozen (-18°C) Storage Then Thawed at Months 0, 1, 2, 3, 4, 5, and 6.

4.3.2 Ash Results

For the most part, the ash contents obtained in this study did not exhibit significant difference between the boiled and steamed crawfish samples in refrigerated storage. The only day in which there was a significant difference was on day nine where the ash content of the steamed sample was higher ($p<0.05$) than that of the boiled sample, as shown in Table 6. The difference in ash content between day zero and eleven are slight and could simply be due to the inherent variation in biological systems such as crawfish. As Sidwell (1981) reported, the range for percent ash in an unspecified species of raw crawfish was 0.7-3.6% which indicates quite a large range of natural variation. The average ash contents of the samples in refrigerated storage were 1.26 percent and 1.31 percent (tail meat with hepatopancreas on a wet weight basis) for the boiled treatment and for the steamed treatment, respectively. The ash contents reported by

Nadarajah and others (2013) and USDA (2014) was 1.2%, which are very close to the values in this study. A study by Moody and Culley (1991) had similar results, an ash content of 1.5%. The values for percent ash content of the refrigerated samples (boiled and steamed) are shown in Table 6.

Table 6. Percent (%) Ash of Peeled Crawfish Tail Meat Stored Under Refrigeration (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	1.30 ± 0.10^{BCD}	1.56 ± 0.16^{A}	1.15 ± 0.03^{E}	1.25 ± 0.08^{BCDE}	1.21 ± 0.03^{BCDE}	1.17 ± 0.06^{DE}	1.19 ± 0.08^{CDE}
Steamed	1.25 ± 0.04^{BCDE}	1.56 ± 0.04^{A}	1.23 ± 0.12^{BCDE}	1.31 ± 0.02^{BC}	1.28 ± 0.03^{BCDE}	1.33 ± 0.01^{B}	1.19 ± 0.08^{CDE}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The percent ash contents of the boiled and steamed samples in frozen storage (samples were thawed prior to measurement) were slightly higher, on average, than the samples in refrigerated storage. The boiled samples in frozen storage had an average ash content of 1.22%; the steamed samples had an average of 1.32% ash. This might indicate more leaching of minerals in the boiled samples compared to that of the steamed samples. Blanching, or boiling, of foods has a tendency to cause leaching of vitamins and minerals, steaming has the benefit of less leaching than blanching/boiling (Reddy and Love, 1999). The values for percent ash in boiled and steamed crawfish tails that were frozen are in Table 7. Month one was the only interval in which there was a significant difference between the ash contents of the boiled and steamed samples. The ash content of the steamed sample (1.40%) was higher ($p>0.05$) than that of the boiled sample (1.01%).

Table 7. Percent (%) Ash of Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0,1,2,3,4,5, and 6.

	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	1.29 ± 0.10^{ABC}	1.01 ± 0.39^{D}	1.31 ± 0.04^{ABC}	1.15 ± 0.02^{CD}	1.21 ± 0.02^{BC}	1.22 ± 0.04^{BC}	1.33 ± 0.00^{ABC}
Steamed	1.25 ± 0.04^{ABC}	1.40 ±0.05^{AB}	1.22 ± 0.08^{BC}	1.21 ± 0.07^{BC}	1.40 ± 0.07^{AB}	1.34 ± 0.03^{ABC}	1.44 ± 0.02^{A}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

Figure 12 illustrates that the steamed samples generally had increased ash values compared to boiled samples through both refrigerated and frozen storage and that those differences were slight and not necessarily meaningful.

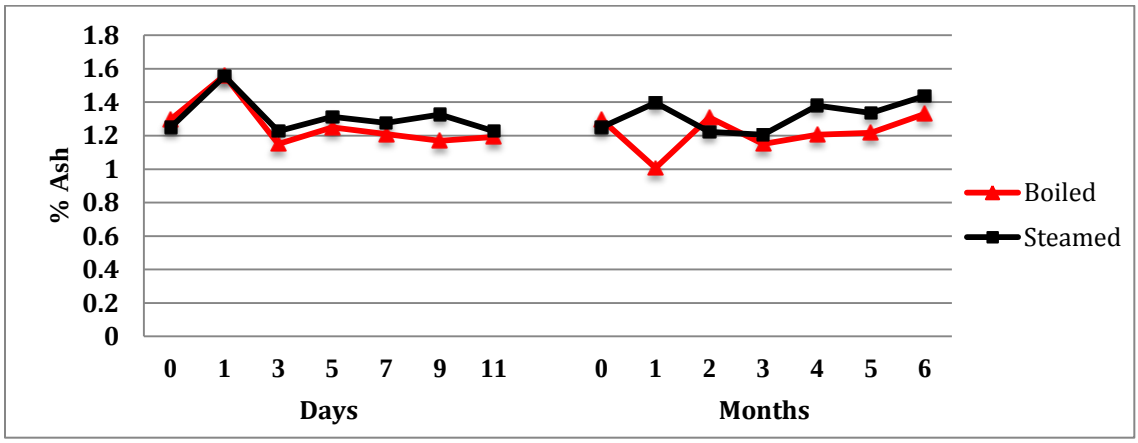


Figure 12. Ash Values (%) for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0,1,3,5,7,9,11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5,and 6.

4.3.3 Protein Results

There were significant differences in protein content between the boiled and steamed crawfish for every same day data point of refrigerated storage. At every point except day seven, the protein content of the boiled samples are higher ($p<0.05$) than that of the steamed samples.

On day seven, the protein content of the steamed samples was significantly greater than that of the refrigerated samples. Although there was significant difference at every measurement interval, the actual differences were small. The average percent protein for boiled samples during refrigerated storage was 15.89% while the average percent protein for steamed samples was 15.30%. These values are slightly lower than the USDA and Sidwell percent protein observations, which were 16.77% and 16.3% respectively, but higher than the percent protein content obtained by (Nadarajah and others, 2013) which was 14.4%. The values observed in this study may have been lower in comparison to Sidwell's results because in this study, the hepatopancreas was kept attached which may have reduced the percentage of protein present. Overall, the protein contents of all data points over time for the boiled samples were different ($p<0.05$). The data for the steamed samples over time were also different ($p<0.05$) except for days seven and nine which were not significantly different from each other. Again, although there were significant differences in protein content, the differences were small.

For both the boiled and steamed samples, there was an overall decrease in percent protein from day zero to day eleven. This may be due to increased microbial counts and the hydrolysis and consumption of free amino acids and other soluble non-nitrogenous substance in the crawfish that serve as nutrients for microbial growth (Zeng and others 2005). The averages for protein content of the boiled meat samples and of the steamed samples (15.89% and 15.30%, respectively) were not greatly different. The results and statistical differences for refrigerated storage of boiled and steamed crawfish are presented in Table 8.

Table 8. Percent (%) Protein of Peeled Crawfish Tail Meat Stored Under Refrigeration (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	16.32 ± 0.04 ^{C}	16.46 ± 0.06 ^{B}	16.93 ± 0.04 ^{A}	15.06 ± 0.00 ^{G}	14.95 ± 0.07 ^{H}	16.23 ± 0.04 ^{D}	15.27 ± 0.05 ^{F}
Steamed	16.15 ± 0.03 ^{D}	15.34 ± 0.06 ^{F}	16.02 ± 0.06 ^{E}	14.56 ± 0.10 ^{I}	15.07 ± 0.04 ^{G}	15.05 ± 0.02 ^{G}	14.91 ± 0.10 ^{H}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The protein content of the boiled samples during frozen storage were higher ($p<0.05$) than steamed samples at every month except month six. The average percent protein for boiled samples was 16.9% and for the steamed samples, 16.3%. There was a distinct difference between the protein content of the frozen samples and the refrigerated samples. The frozen values are larger, by almost one percent and are almost on point with the USDA (2014) value of 16.77%. These values also fall within the range (11.9 - 24.1%) for percent protein in raw samples reported by Sidwell (1981) in Table 3.

Table 9. Percent (%) Protein of Thawed, Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	16.32 ± 0.04^{EF}	17.31 ± 0.06^{A}	16.52 ± 0.03^{D}	16.71 ± 0.03^{C}	16.21 ± 0.19^{FG}	17.26 ± 0.02^{A}	16.51 ± 0.04^{D}
Steamed	16.15 ± 0.03^{GH}	16.02 ± 0.05^{HI}	16.32 ± 0.06^{EF}	16.44 ± 0.16^{DE}	15.90 ± 0.04^{I}	16.37 ± 0.05^{E}	16.90 ± 0.01^{B}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

There are slight differences in the protein content between boiled and steamed crawfish during refrigerated and frozen storage. The difference could simply arise from natural variation, and Figure 12 depicts the protein content of both boiled and steamed meat in refrigerated and in frozen storage. The protein content of frozen samples tend to be higher than that of the refrigerated samples. The differences may be significant but not necessarily meaningful in a commercial sense, and is not indicative of no distinct benefit to one cooking method over the other in protein content after cooking.

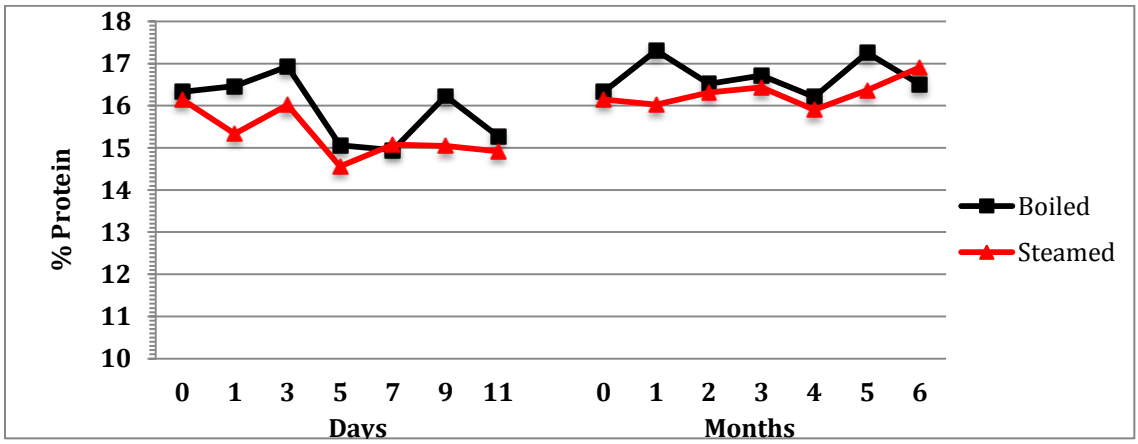


Figure 13. Protein Values (%) for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0,1,3,5,7,9,11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

4.3.4 Fat Results

The percent fat values obtained in this study (2.12 to 3.76) are higher than literature values. For example, the USDA (2014) lists the percent fat as 1.2%, Sidwell (1981) gave 0.8%, and Nadarajah and others (2013) reported the percent fat to be 1.5%. In the current study, every value for fat during either refrigerated or frozen storage, boiled or steamed, was higher than these previous studies. This may be due to the inclusion of the hepatopancreas (fat) with the tail meat in the present study. In Louisiana, crawfish are traditionally consumed with the fat (Horst 2010). During this study, the hepatopancreas was kept attached to the tail meat to mimic the way that

crawfish are commonly consumed and in whole boil, it would remain attached.

Most studies delineating the fat content of crawfish tail meat do not include the hepatopancreas. The hepatopancreas is roughly 30% fat by weight (Reames, 2010). Therefore, the fat content of crawfish with hepatopancreas would be expected to be higher than crawfish tail meat without hepatopancreas. In the current study, the average fat content of the crawfish tail meat (wet weight basis) was 2.73% for the boiled and refrigerated treatment and 2.96% for the steamed and refrigerated treatment.

Table 10. Percent (%) Fat of Peeled Crawfish Tail Meat Under Refrigerated Storage (3°C) at Days 0,1,3,5,7,9, and 11.

	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	2.99 ± 0.04 **CDE**	2.91 ± 0.04 **DE**	2.58 ± 0.08 **F**	2.27 ± 0.13 **G**	2.24 ± 0.06 **G**	3.27 ± 0.07 **BC**	2.88 ± 0.11 **DE**
Steamed	2.76 ± 0.16 **DEF**	2.75 ± 0.27 **EF**	3.04 ± 0.03 **CD**	2.12 ± 0.11 **G**	3.41 ± 0.27 **A**	2.90 ± 0.08 **DE**	3.76 ± 0.06 **A**

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The average fat content of the crawfish tail meat (wet weight basis) was 3.01% for the boiled and frozen treatment and 3.22% for the steamed and frozen treatment. The average fat content of the frozen crawfish was higher for both the boiled and steamed treatments than for the same treatments with refrigerated storage. This might be explained by the reduced moisture in the frozen samples compared to the refrigerated samples. The variation is much more pronounced for the steamed samples than for the boiled samples.

Table 11. Percent (%) Fat of Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0,1,2,3,4,5, and 6.

	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	2.99 ± 0.04 DEF	3.10 ± 0.0 DE	2.72 ± 0.08 H	3.13 ± 0.10 CDE	3.00 ± 0.08 DEF	2.95 ± 0.01 EFG	3.15 ± 0.04 CDE
Steamed	2.76 ± 0.16 GH	3.07 ± 0.04 DEF	3.34 ± 0.06 BC	3.87 ± 0.10 A	2.86 ± 0.03 FGH	3.21 ± 0.26 BCD	3.42 ± 0.11 B

LS-Means ± SD of 2 measurements at each time period. LS-Means with same letter are not different ($P < 0.05$)

Figure 14 illustrates that steamed samples contained slightly more fat as a whole, and that the differences are small and thus may not be commercially meaningful.

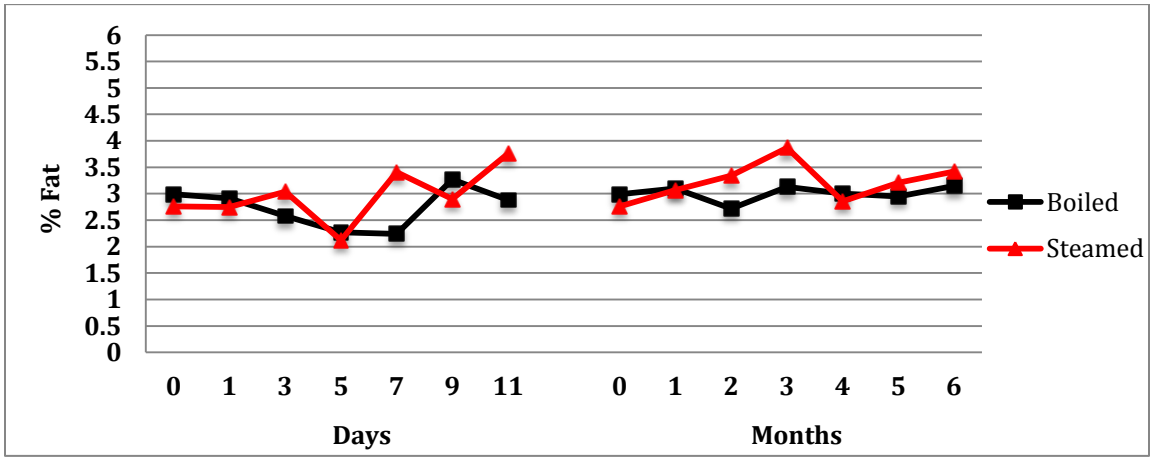


Figure 14. Fat Values (%) for Boiled and for Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0,1,3,5,7,9,11 and Frozen Storage (-18°C) at Months 0,1,2,3,4,5, and 6.

4.4 Microbiological Results

There were no *E. coli*/coliforms present in any of the water samples or any of the boiled or steamed crawfish at any time. There was no presence of *E. coli* in the raw crawfish, but there was an average coliform count of 295 CFU/g. This suggests that the cook procedure for both boiling and steaming was adequate to kill these organisms. An APC count of log 5.7 CFU/g is

suggested by ICMSF (1986) as the upper limit of acceptability and, in this study, taken as the upper limit for shelf life determination.

As seen in Table 20, the boiled and steamed samples in refrigerated storage did not exceed the limit of log5.7 CFU/g until after day three. Both the boiled and steamed samples exceeded the chosen limit by day 5, the data point immediately after day 3. Therefore the acceptable shelf life of refrigerated crawfish, either boiled or steamed, was taken as three days. It is clear that the aerobic bacteria in the steamed samples started logarithmic growth after day one whereas the aerobic bacteria in boiled samples did not exhibit logarithmic growth until after day three. The steamed samples almost exceeded 5.7 log CFU/g on day three. It is not clear why the difference in APC occurred. Further study with analyses conducted on all days may provide better insight.

Table 12. Aerobic Plate Counts (log CFU/g) Values for Peeled Crawfish Tail Meat Under Refrigerated Storage (3°C) at Days 0,1,3,5,7,9, and 11.

	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	3.03 ± 2.87^{C}	3.10 ± 2.87^{C}	3.03 ± 2.91^{C}	6.10 ± 5.36^{C}	6.12 ± 5.05^{C}	8.10 ± 7.08^{B}	8.01 ± 6.94^{B}
Steamed	2.57 ± 2.53^{C}	2.78 ± 1.97^{C}	5.46 ± 4.89^{C}	6.22 ± 5.08^{C}	TNC*	8.62 ± 7.53^{A}	8.65 ± 8.00^{A}

LS-Means ± SD of 4 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$)

* TNC = Too Numerous to Count

The APC counts were essentially constant during frozen storage and below 5.7 log CFU/g at all data points as shown in Table 21 which would be expected during frozen storage at -18°C. This suggests that the shelf life, based upon APC counts, of crawfish in frozen storage is at least 6 months. There were differences ($p<0.05$) in APC counts within as well as between the boiled and the steamed samples, but the differences were small and suggest that the differences

may not be commercially meaningful. Figure 19 illustrates the logarithmic growth of aerobic bacteria in refrigerated storage and the lack of growth and minimal aerobic counts for the frozen samples.

Table 13. Aerobic Plate Counts (log CFU/g) Values for Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	3.02 ± 2.87^{A}	2.27 ± 2.18^{D}	2.48 ± 1.98^{D}	2.90 ± 2.19^{ABC}	2.69 ± 2.00^{BCD}	2.78 ± 2.00^{BCD}	2.79 ± 2.77^{BCD}
Steamed	2.57 ± 2.53^{CD}	2.70 ±2.02^{BCD}	2.69 ±1.83^{BCD}	2.95 ± 2.56^{AB}	2.56 ± 1.70^{CD}	2.92 ± 2.41^{AB}	3.04 ± 2.41^{A}

LS-Means ± SD of 4 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

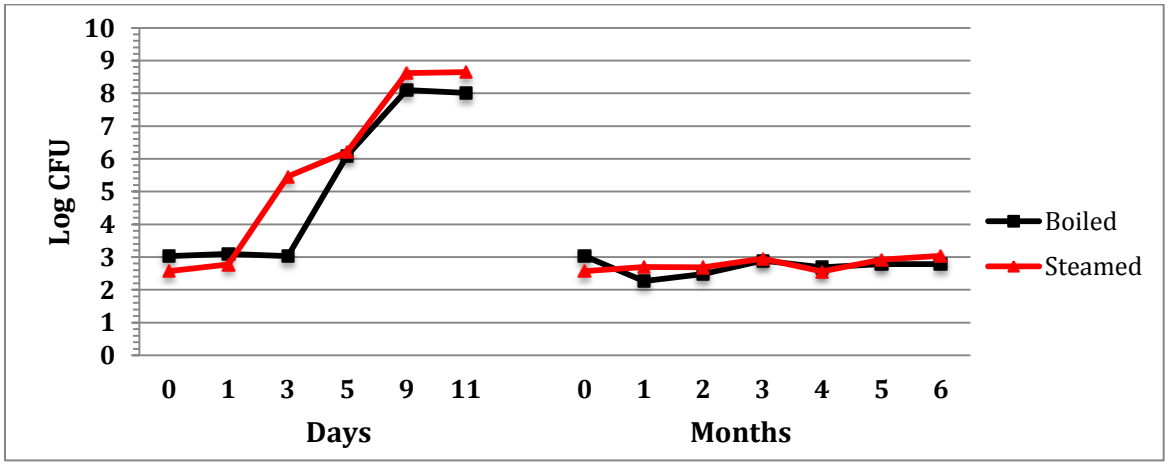


Figure 15. Aerobic Plate Counts (Log CFU) for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0, 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

4.5 TBARS Results

TBAR values increased during refrigerated storage for both boiled and steamed samples but the level of TBARS measured at the end of the refrigerated storage was lower than anticipated. It was anticipated that the values would be higher than previous studies because previous studies were conducted on crawfish in which the samples had the hepatopancreas

removed and the tail deveined. The crawfish prepared in this study did not. The hepatopancreas is roughly 30% fat by weight which would result in a larger fat content than would be obtained for tail meat with the hepatopancreas and vein removed (see Fat. 4.3.4). It was postulated that more fat would result in higher TBARS values. Surprisingly, the TBARS values obtained in this study were lower than literature values. Cremades and others (2011) conducted a study on refrigerated storage of crawfish tail meat in which they obtained an average TBARS value of 2.24 mg MDA/kg after 7 days of refrigerated storage.

Table 14. TBARS Values (mg MDA/kg of tail meat) for Peeled Crawfish Tail Meat Under Refrigeration Storage (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	0.19 ± 0.02 **FG**	0.15 ± 0.01 **H**	0.24 ± 0.00 **E**	0.33 ± 0.03 **CD**	0.34 ± 0.01 **C**	0.42 ± 0.04 **B**	0.45 ± 0.02 **B**
Steamed	0.16 ± 0.01 **GH**	0.14 ± 0.02 **H**	0.22 ± 0.00 **EF**	0.29 ± 0.03 **D**	0.30 ± 0.04 **D**	0.32 ± 0.01 **CD**	0.53 ± 0.03 **A**

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

During refrigerated storage, TBARS values of the boiled and steamed samples gradually increased from day one to day eleven. It was unexpected that day zero values would be more than day one values. One possible explanation for this behavior is that on day zero, many analyses were conducted and the samples for the TBAR analysis had remained at room temperature slightly longer than on the other days of the study. Except for the day zero anomalies, the two cooking methods exhibited steady increases in TBARS values, as had been expected, through storage with boiled samples having slightly higher values than the steamed samples on all days except day 11. The average TBARS value for boiled samples in refrigerated

storage was 0.30 mg MDA/kg with a maximum value of 0.45 mg MDA/kg reached on day eleven. The average TBARS value for steamed samples in refrigerated storage was 0.28 mg MDA/kg. A maximum value of 0.53 mg MDA/kg was reached on day 11. According to Treece and others (1985), 1.5 mg MDA/kg of TBARS is the point at which humans can detect any off flavors and 3.0 mg MDA/kg of TBARS is the level at which crawfish are considered rancid and not desirable for consumption.

According to these criteria, the fat in the crawfish tail meat and hepatopancreas was not rancid at any time during the course of the study. However, it was noticed that the crawfish in the last five to six days of the refrigerated study had an odor that was unpleasant. It may also be interesting to note that Cremades and others (2011) identified much higher levels of TBARS of crawfish in refrigerated storage compared to the values obtained in this study and that their initial TBARS value before any storage was 2.08 mg MDA/kg which is higher than the 1.5 mg MDA/kg suggested by Treece and others (1985) as the level at which off-flavors are detectable.

Table 15. TBARS Values (mg MDA/kg of tissue) for Peeled Crawfish Tail Meat Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.19 ± 0.02 **F**	0.22 ± 0.02 **DEF**	0.16 ± 0.02 **G**	0.21 ± 0.01 **DEF**	0.23 ± 0.01 **CD**	0.23 ± 0.01 **CDE**	0.32 ± 0.03 **A**
Steamed	0.16 ± 0.01 **G**	0.25 ± 0.00 **BC**	0.20 ± 0.00 **EF**	0.22 ± 0.01 **CDE**	0.19 ± 0.01 **F**	0.26 ± 0.04 **B**	0.22 ± 0.02 **CDE**

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letters are not different ($P < 0.05$).

Frozen storage TBARS values for both boiled and steamed samples, although having statistically significant differences, were essentially stable in contrast to the TBARS values of the refrigerated samples, which steadily increased from day 1 to day 11 of refrigerated storage. The

average TBARS value for boiled samples in frozen storage was 0.22 mg/kg with a maximum value of 0.32 mg MDA/kg reached at month six. The average TBARS value for steamed samples in frozen storage was 0.21 mg MDA/kg with a max value of 0.26 mg MDA/kg that occurred at month five. Amr and Rutledge (1980) observed an average TBARS value of 4.00 mg MDA/kg over the course of ten months of frozen storage, with a maximum value at month ten of 5.60 mg MDA/kg. These values were for crawfish tails containing the hepatopancreas.

Amr and Rutledge (1980) evaluated TBARS for tail meat (with and without hepatopancreas attached) from whole crawfish that had been frozen through 10 months of storage (-18°) then thawed for analysis. They used a similar method to that in the present study to conduct their TBARS analyses. It was thought that the values observed in this study would be comparable to theirs, but their TBARS values of 1.53, 3.90, 3.08, 4.36, 5.51, and 5.60 mg/kg at months 0, 2, 4, 6, 8, and 10 respectively were several times higher than the values observed in the present study.

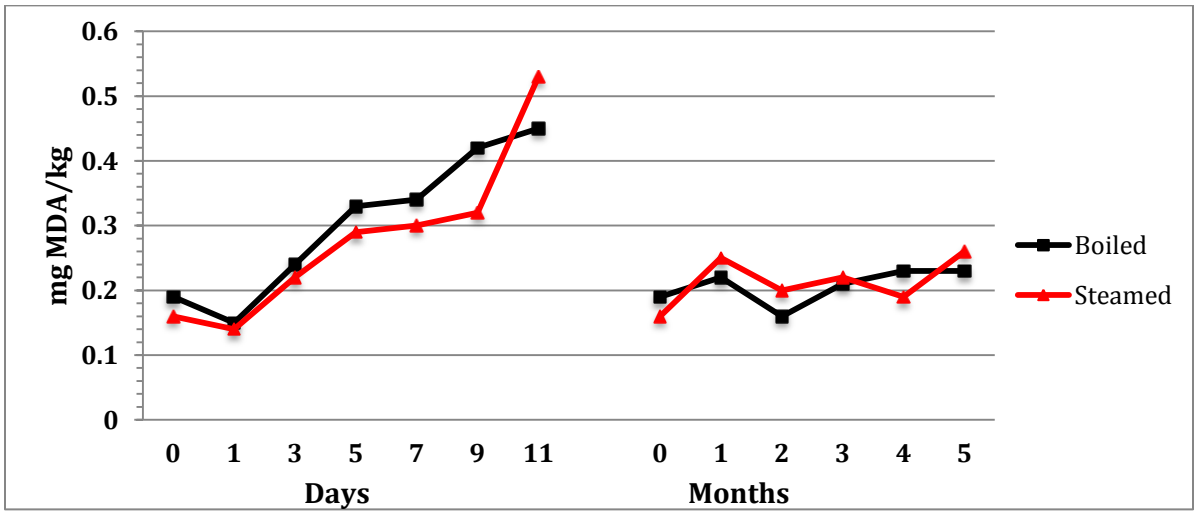


Figure 16. TBARS Values (mg MDA/kg of tissue) for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0, 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

The values for TBARS, either boiled or steamed, did not approach the flavor detection point (1.5mg/kg; Treece and others, 1985). The differences from one cooking method to the other are small, and probably would not have an appreciable effect on the commercial viability of either product.

4.6 Texture

Texture analyses were conducted on crawfish tail meat with adhering hepatopancreas over the course of refrigerated and frozen storage. It was unclear what the effect on the texture, in particular, toughness and mushiness through storage would be. Toughening might occur due to moisture loss through the course of storage, but texture could deteriorate or become mushy due to residual activity of native proteinases in the hepatopancreas or tail that had not been deactivated by the thermal treatment (cooking) process. Peak shear force and work values were chosen to characterize the texture. Table 14 shows the peak shear force for boiled and steamed peak shear force values for crawfish during eleven days of refrigerated storage.

Table 16. Peak Shear Force (kg) for Peeled Crawfish Tail Meat Under Refrigerated Storage (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	23.61 ± 0.88 ^{ABCD}	24.54 ± 2.13 ^{ABC}	26.76 ± 2.88 ^{A}	22.29 ± 1.20 ^{BCDE}	23.55 ± 0.538 ^{BCD}	25.39 ± 3.44 ^{AB}	23.26 ± 0. 89 ^{BCD}
Steamed	22.90 ± 3.09 ^{BCD}	21.25 ± 1.48 ^{DE}	22.17 ± 1.96 ^{CDE}	22.21 ± 1.50 ^{BCDE}	24.08 ± 0.79 ^{ABCD}	22.50 ± 1.63 ^{BCD}	19.19 ± 1.44 ^{E}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The peak shear force (kg) for the boiled samples in refrigerated storage increased from day zero to day three even though the values were not different ($p<0.05$), there was greater

variability from day three to eleven. The trend was somewhat similar for steamed samples, but the final value for steamed samples was lower than the boiled on day 11. The steamed shear force values are statistically similar but the shear force value on day eleven was the lowest. These results follow previous results by Marshall (1985) which suggested that proteolytic enzymes and an increased bacterial presence through the refrigerated storage caused the deterioration in the texture of the crawfish. However, day seven variations in texture may also be due to the inherent nature of crawfish and the Kramer analysis. The uniformity of the sample and direction of the muscle fibers plays a role in the outcome of the analysis (Szczesniak and Torgeson, 1965). That the crawfish were placed in the Kramer cell at random and did not completely cover the bottom of the cell in the present study may have had an impact. The weight of crawfish added to the cell was kept constant for all samples, however the number of crawfish changed since the weights of individual crawfish tails varied.

Table 17. Peak Shear Force Values (kg) for Peeled Crawfish Tail Meat Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	23.61 ± 0.88 ^{F}	28.27 ± 2.46 ^{ABC}	27.14 ± 2.02 ^{ABCDE}	27.13 ± 1.73 ^{ABCDE}	29.68 ± 2.66 ^{A}	27.81 ± 0.37 ^{ABCD}	28.78 ± 1.93 ^{AB}
Steamed	22.90 ± 3.09 ^{F}	24.08 ± 1.9 ^{EF}	24.72 ± 0.79 ^{DEF}	25.24 ± 0.99 ^{CDEF}	25.76 ± 2.83 ^{BCDEF}	27.04 ± 1.65 ^{ABCDE}	29.15 ± 0.85^{A}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The peak shear force (kg) for the frozen crawfish tail meat with attached hepatopancreas was more consistent than for the refrigerated samples. On average, the boiled samples were tougher than the steamed samples. However, at month six, the steamed sample had a higher peak shear force (kg) than the boiled sample. Since the microbiological activity during the frozen

storage was negligible, other factors were at work in caused the increase in toughness compared to that of the refrigerated samples, most likely due to moisture loss. Again, when considering Figure 17, there was an apparent difference between boiled and steamed samples but these differences were not likely meaningful.

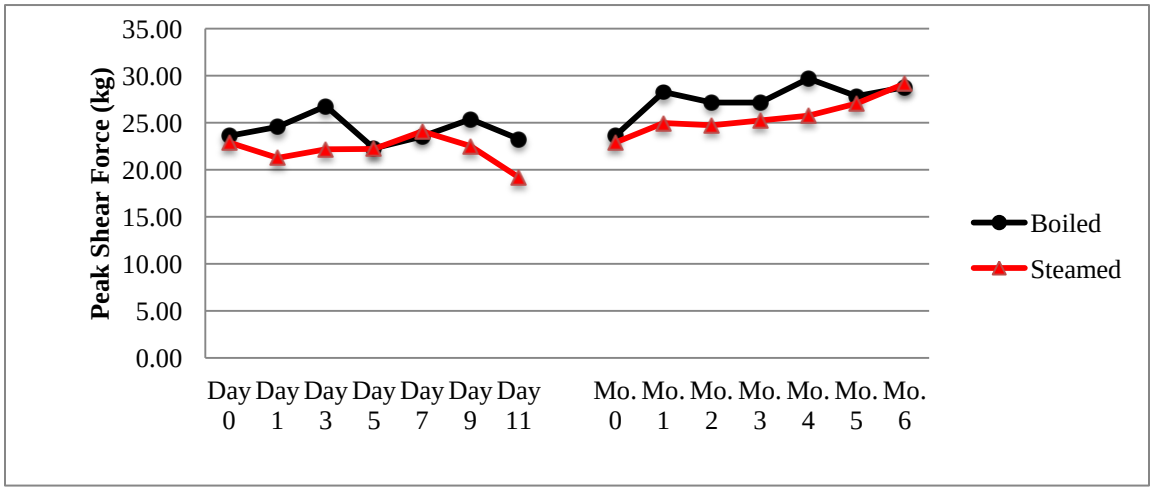


Figure 17. Peak Shear Force (kg) for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0, 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

The work of shearing was chosen as a measure of toughness perceived when biting into a food product by measuring the force needed to shear the sample as a function of time. The results of the work of shearing mimic the values of peak shear force, which might be expected considering the measurements were taken simultaneously. Values for work of shearing of boiled and frozen crawfish tails through refrigerated storage are in Table 16 and through frozen storage in Table 17.

Table 18. Work Values (kg*m/s) for Peeled Crawfish Tail Meat Under Refrigeration Storage (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	108.02 ± 8.77 ^{BCDE}	110.30 ± 15.54^{ABCD}	126.59 ± 5.36^{A}	106.59 ± 13.62 ^{BCDE}	106.61 ± 5.82 ^{BCDE}	111.41 ± 3.57 ^{ABC}	103.10 ± 9.11 ^{BCDEF}
Steamed	112.69 ± 15.05 ^{AB}	92.52 ± 1.59 ^{EF}	101.43 ± 11.28^{BCDEF}	94.18 ± 12.09^{DEF}	112.76 ± 7.19^{AB}	95.16 ± 8.77 ^{CDEF}	86.60 ± 10.40 ^{F}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

Table 19. Work Values (kg*m/s) for Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	108.02 ± 8.77 ^{BCD}	118.03 ± 14.13^{BC}	115.27 ± 11.80^{BC}	109.46 ± 11.96 ^{BCD}	137.42 ± 3.71 ^{A}	125.09 ± 3.37 ^{AB}	112.32 ± 7.79 ^{BC}
Steamed	112.69 ± 15.05 ^{BC}	84.86 ± 4.45 ^{E}	102.86 ± 3.40 ^{CDE}	91.36 ± 5.39 ^{DE}	119.36 ± 18.49 ^{ABC}	120.60 ± 20.44 ^{ABC}	116.12 ± 5.55 ^{BC}

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$)

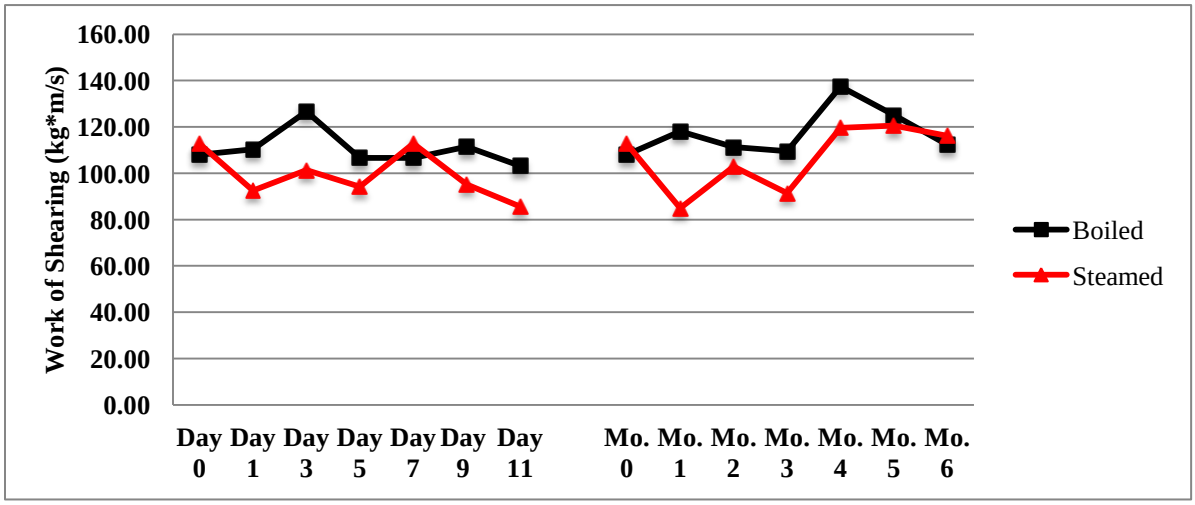


Figure 18. Work of Shearing (kg*m/s) for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0, 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

4.7 pH Results

Table 20. pH Values for Peeled Crawfish Tail Meat Under Refrigeration Storage (3°C) at Days 0, 1, 3, 5, 7, 9, and 11.

	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	8.10 ± 0.00 EF	8.20 ± 0.00 CD	8.10 ± 0.00 EF	8.17 ± 0.06 DE	8.40 ± 0.00 A	8.27 ± 0.06 BC	8.03 ± 0.06 F
Steamed	8.30 ± 0.00 B	8.20 ± 0.00 CD	8.10 ± 0.00 EF	8.13 ± 0.06 DE	8.17 ± 0.06 DE	8.03 ± 0.06 F	7.77 ± 0.06 G

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

There was no general trend for the pH of the boiled crawfish during refrigerated storage. For both the boiled and steamed samples, the pH increased and decreased during storage to a final pH value of 7.77 on day 11 for the steamed samples and 8.03 for the boiled samples. The trend of increased pH for boiled and steamed samples through the refrigerated storage can be attributed to an increased production of basic compounds such as ammonia and other biogenic amines (Masniyom and others 2002; Laursen and others 2006). In frozen storage, there were also significant pH decreases from month 0 to month 6 (which had the lowest pH values) for both boiled and steamed crawfish tail meat in frozen storage which are present in table 19.

Table 21. pH Values for Peeled Crawfish Tail Meat in Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	8.10 ± 0.00 DE	8.20 ± 0.00 BC	8.27 ± 0.06 AB	8.23 ± 0.06 ABC	8.20 ± 0.00 BC	7.87 ± 0.06 FG	7.87 ± 0.06 FG
Steamed	8.30 ± 0.00 A	8.03 ± 0.06 E	8.20 ± 0.00 BC	8.17 ± 0.06 CD	8.20 ± 0.00 BC	7.93 ± 0.06 F	7.83 ± 0.06 G

LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

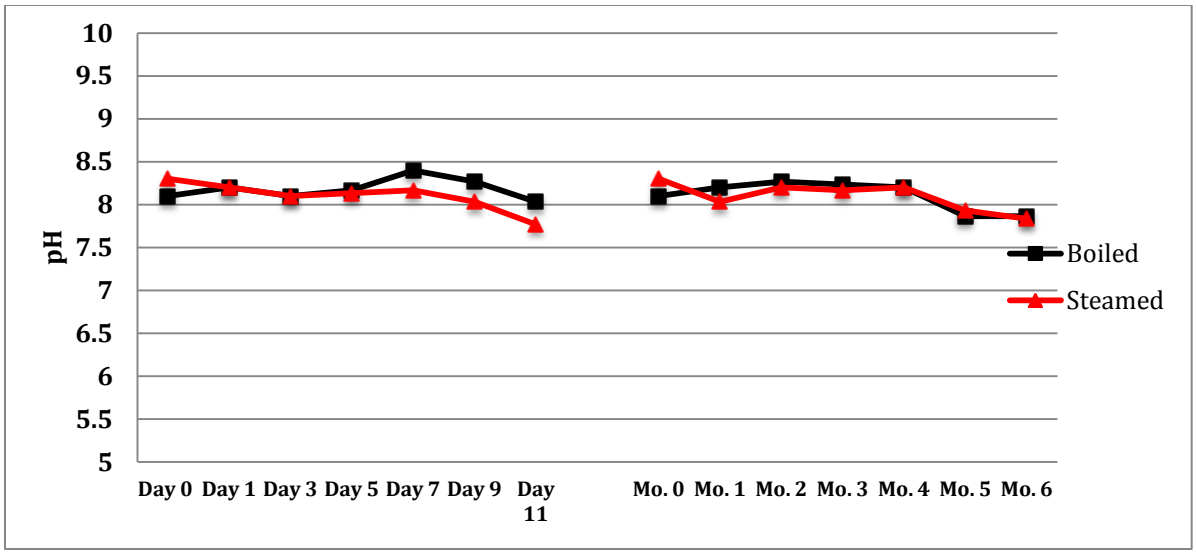


Figure 19. pH Values for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0, 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

4.8 Color Results

The results of the color analyses for lightness (L*), green/red (a*), and blue/yellow (b*) showed only slight differences throughout refrigerated and frozen storage. For boiled samples during refrigerated storage, the L* values were similar ($p<0.05$) except for the difference ($p<0.05$) in L* between days seven and eleven which were the days in which the lightness values observed were the lowest and highest as shown in Table 20.

Table 22. L* Values for Peeled Crawfish Tail Meat During Refrigerated Storage (3°C) on Days 1, 3, 5, 7, 9, and 11.

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	53.30 ± 6.26^{BC}	54.06 ± 3.45^{ABC}	53.23 ± 6.39^{BC}	50.61 ± 6.23^{C}	54.73 ± 4.28^{ABC}	57.84 ± 6.70^{AB}
Steamed	53.65 ± 5.57^{BC}	52.91 ± 5.85^{BC}	51.34 ± 3.85^{C}	55.10 ± 3.91^{ABC}	56.06 ± 7.20^{ABC}	59.33 ± 10.64^{A}

LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

For the steamed samples, lightness values slowly decreased from day one to day five, then increased to day eleven. There was no significant difference ($p<0.05$) between the two

treatments at each individual day. Also, as shown in figure 20, the two cooking methods shared the same trend. The small differences in L* values suggest that there is no meaningful difference for L* values between the two cooking methods.

Table 23. L* Values for Peeled Crawfish Tail Meat During Frozen Storage (-18°C) on Months 1, 2, 3, 4, 5, and 6.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	52.32 ± 4.28^{AB}	54.45 ± 5.76^{AB}	53.74 ± 6.66^{AB}	56.60 ± 7.43^{A}	48.63 ± 5.91^{B}	48.58 ± 6.92^{B}
Steamed	52.12 ± 9.18^{AB}	53.11 ± 4.45^{AB}	52.46 ± 6.58^{AB}	52.84 ± 7.29^{AB}	49.86 ± 6.72^{B}	54.23 ± 7.71^{AB}

LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The L* values of the boiled samples through frozen storage were all similar except that month four was higher ($p<0.05$) than months 5 and 6. As with the refrigerated samples through eleven days of storage, the boiled and steamed frozen crawfish L* values were not different ($p<0.05$) from each other at any month. These L* values through refrigerated storage were slightly higher than the samples in frozen storage.

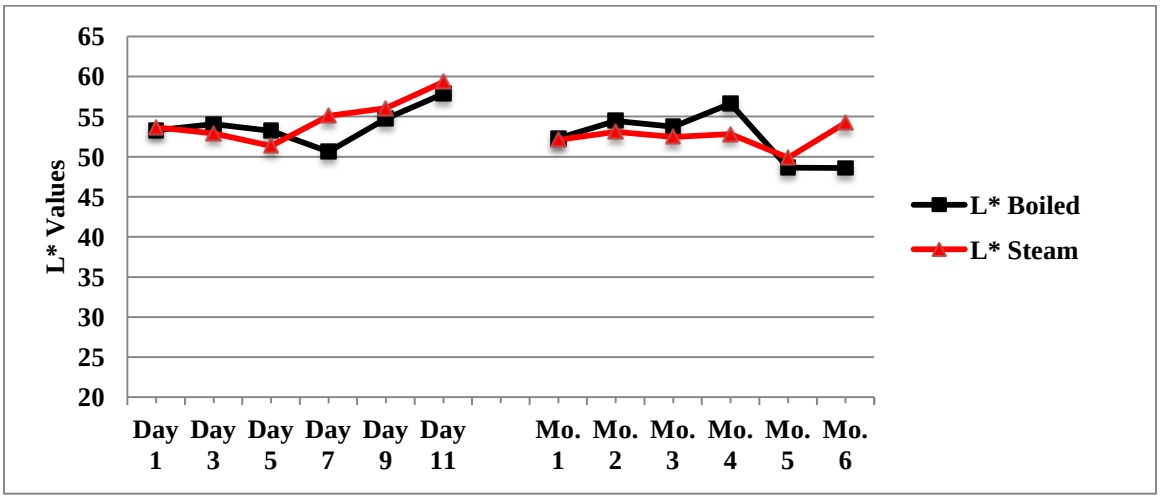


Figure 20. Lightness (L*) Values for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 1, 2, 3, 4, 5, and 6.

The a* values or green/red scale of color showed little difference ($p<0.05$) for boiled crawfish samples throughout refrigerated storage with day three having the highest ($p<0.05$) a* value. Values for the steamed samples fluctuated and on day 11 the steamed samples had much smaller values L* values than the boiled samples. On day 11, the steamed a* value was 22.91 which is the lowest value obtained, was a sharp decrease from day nine and significantly less than the 31.06 value which was observed for the boiled samples.

Table 24. a* Values for Peeled Crawfish Tails Under Refrigerated Storage (3°C) on Days 1, 3, 5, 7, 9, and 11.

	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	32.02 ± 6.39^{BCD}	35.52 ± 5.73^{AB}	32.58 ± 4.63^{BCD}	31.65 ± 3.74^{BCD}	29.35 ± 3.63^{CD}	31.06 ± 5.77^{BCD}
Steamed	34.18 ± 8.04^{ABC}	38.23 ± 6.94^{A}	31.47 ± 5.27^{BCD}	27.35 ± 4.03^{DE}	29.35 ± 3.63^{DE}	22.91 ± 10.45^{E}

*LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

The a* values for the boiled and steamed frozen samples were all similar ($p<0.05$) at each month except month six. At month six, the a* value of the boiled samples were larger than that of the steamed samples. This was similar to what occurred during refrigerated storage with certain exceptions. The a* values increased for boiled samples from month one to month six frozen storage. The steamed samples a* values fluctuated throughout frozen storage with the only significant difference from month to month occurring from month one to month two during which the a* values significantly increased. Figure 17 illustrates an apparent overall decrease in a* values over time from refrigerated samples while the frozen values fluctuated with a tendency to increase with time of storage.

Table 25. a* Values for Peeled Crawfish Tail Meat Under Frozen Storage (-18°C) at Months 1, 2, 3, 4, 5, and 6.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	25.80 ± 4.08^{BC}	26.58 ± 5.04^{BC}	27.24 ± 7.27^{BC}	24.55 ± 8.27^{BC}	30.15 ± 6.23^{AB}	32.93 ± 5.77^{A}
Steamed	23.82 ± 8.18^{C}	30.12 ± 3.48^{AB}	27.21 ± 6.21^{BC}	26.99 ± 4.83^{BC}	28.93 ± 7.80^{ABC}	25.39 ± 7.40^{BC}

LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letter are no different ($P < 0.05$).

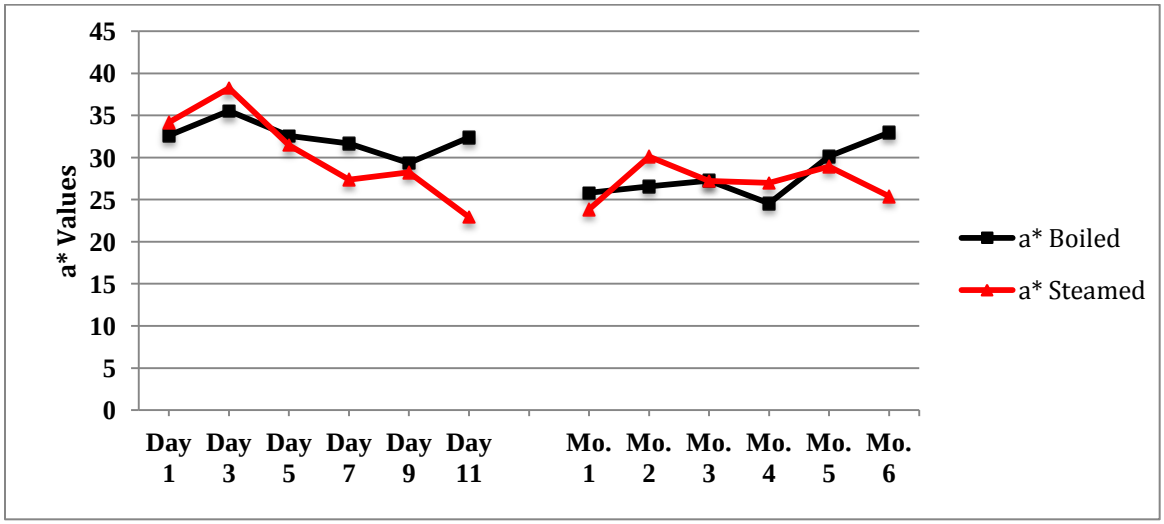


Figure 21. Red/green (a*) Values for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 1, 2, 3, 4, 5, and 6.

The b* values for refrigerated boiled samples (blue/yellow color scale) fluctuated during the eleven days of refrigerated storage. The boiled refrigerated b* value ($p<0.05$) increased from day one to day three, then decreased from day three to day seven when the lowest value of 22.91 occurred. The b* values then steadily increased to day eleven. It is not understood what caused the fluctuation in the b* values during the refrigerated storage. For the steamed samples, there

was a sharp increase in the b* value from day one to day three and then followed by a decrease and a plateau in values towards day seven, nine, and eleven.

Table 26. b* Values for Peeled Crawfish Tail Meat Under Refrigerated Storage (3°C) on Days 1, 3, 5, 7, 9, and 11.

	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**
Boiled	32.48 ± 4.66^{C}	37.78 ± 5.99^{AB}	30.78 ± 4.88^{CD}	22.91 ± 3.40^{F}	26.29 ± 4.63^{DEF}	32.35 ± 4.30^{C}
Steamed	34.68 ± 6.31^{BC}	40.24 ± 5.35^{A}	30.10 ± 4.10^{CDE}	26.77 ± 4.98^{DEF}	25.49 ± 3.30^{EF}	27.60 ± 8.46^{DE}

LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letter are not different ($P < 0.05$).

During frozen storage, the b* values were lower than the b* values during refrigerated storage which corresponds to the L* and a* values that were observed. The steamed and boiled samples were significantly the same throughout the six months of storage at every month. The boiled samples showed no ($p<0.05$) change throughout the six months of storage. The steamed samples also showed no significant change through the six months of storage.

Table 27. b* Values for Peeled Crawfish Tails Under Frozen Storage (-18°C) on Days 1, 2, 3, 4, 5, and 6.

	Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	22.57 ± 5.37^{AB}	24.30 ± 5.04^{AB}	24.24 ± 4.73^{AB}	25.60 ± 3.86^{A}	26.12 ± 5.56^{A}	25.59 ± 5.13^{A}
Steamed	20.94 ± 7.84^{B}	25.03 ± 2.22^{AB}	22.50 ± 2.22^{AB}	25.82 ± 2.13^{A}	24.86 ± 4.47^{AB}	25.11 ± 3.78^{A}

*LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letters are not different ($P < 0.05$).

The L*, a*, and b* values all decreased during frozen storage in comparison to their refrigerated samples.

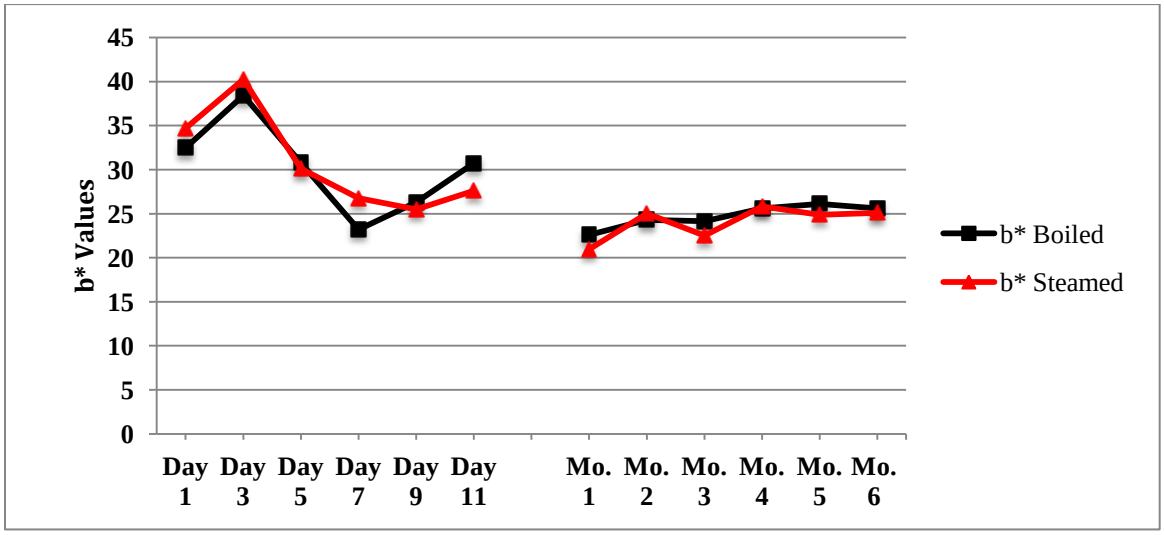


Figure 22. (b*) Values for Boiled and Steamed Peeled Crawfish Tail Meat in Refrigerated Storage (3°C) on Days 0, 1, 3, 5, 7, 9, 11 and Frozen Storage (-18°C) at Months 0, 1, 2, 3, 4, 5, and 6.

4.9 Mineral Results

Mineral analyses were conducted to determine the presence and quantity of 29 different minerals and metals throughout the refrigerated and frozen storage. In this section, only the averages of selected minerals are discussed. The results in total for the mineral analyses are located in the appendix section. The amount of arsenic observed is presented here because of discussions and studies regarding the levels of inorganic and organic arsenic in rice and rice products (Heitkemper and others, 2001; FDA, 2013). Many crawfish are farmed in rice ponds, as were the crawfish in this study. Arsenic levels in the crawfish are of interest because the majority of a crawfish's diet consists of aquatic invertebrates that feed on the detritus matter in the rice

fields/crawfish ponds in which they are farmed. The arsenic levels observed in the crawfish in the present study are higher than that which is approved by the Environmental Protection Agency (EPA) for allowable limits in drinking water. The limit for arsenic in drinking water is 10 parts per billion (ppb) or 0.01 parts per million (ppm). The average arsenic level observed was 0.08 ppm for boiled samples with a range of 0.01-0.15 ppm and an average level of 0.08 ppm for steamed samples with a range of 0.01-0.13 ppm.

As a result of naturally occurring metabolic processes in the biosphere, arsenic occurs as a large number of forms in food (species). Especially in the marine environment arsenic is often determined in higher concentrations of organic forms, up to 50 mg/kg of arsenic on a wet weight basis in some seafood including seaweed, fish, shellfish and crustaceans. In fresh water and in the terrestrial environments arsenic is normally determined in much lower levels (typically 0-20 ug/kg) in crop plants and in livestock. Higher levels may be determined in rice, mushrooms and sometimes in poultry, which is fed fishmeal containing arsenic. The most toxic forms of arsenic are the inorganic arsenic (III) and (V) compounds; the inorganic arsenic trioxide is well known as rat poison, which was also sometimes used for homicide. Methylated forms of arsenic have a low acute toxicity; arsenobetaine, which is the principal arsenic form in fish and crustaceans, is considered non-toxic. In shellfish, mollusks, and seaweed dimethylarsinylriboside derivatives occur ("arsenosugars"), the possible toxicity of which is not known in detail. Only a few percent of the total arsenic in fish is present in inorganic form, which is the only form about which a provisional tolerable weekly intake (PTWI) had been developed by a joint expert committee on food additives (Codex 2014).

Table 28. List of Selected Minerals and Values Observed (ppm of tail meat with adhering hepatopancreas).

Mineral	Boiled Avg. ± SD (ppm)	Boiled Range (ppm)	Steamed Avg. ± SD (ppm)	Steamed Range (ppm)	Other Sources (ppm)
Al	9.67 ± 2.98	4.72 – 13.78	8.54 ± 3.68	4.01 – 16.83	_
Mg	86.08 ± 4.86	78.02 - 90.36	81.76 ± 4.73	70.97 – 87.64	330 (USDA 2014)
Mn	1.04 ± 0.24	0.41 – 1.29	1.06 ± 0.24	0.36 – 1.29	4.2-7.28 (Sidwell 1980)
K	576.32 ± 46.15	641.46 – 494.79	647.13 ± 64.06	535.89 – 738.37	5,000 (Sidwell 1980)
Na	341.98 ± 21.80	300.21 – 364.26	383.51 ± 39.82	325.93 – 431.24	1,820 (Sidwell 1980)
Si	15.77 ± 2.91	11.18 – 21.30	15.71 ± 3.75	10.22 – 21.58	_
B	4.27 ± 0.32	3.73 – 4.77	4.43 ± 0.28	3.84 – 4.85	_
Cu	2.60 ± 0.81	1.48 – 4.47	2.40 ± 0.92	0.73 – 3.98	7-11.21 (Sidwell 1980)
Fe	14.55 ± 2.68	10.32 – 18.61	12.80 ± 2.57	6.53 – 16.22	9-373 (Sidwell 1980)
Ca	416.16 ± 68.71	333.74 – 545.91	371.20 ± 71.10	297.09 – 515.43	650-2,700 (Sidwell 1980)
P	435.74 ± 51.60	346.44 – 506.66	426.01 ± 64.05	308.32 – 524.12	1,010-1,920 (Sidwell 1980)
Zn	5.92 ± 0.72	4.08 – 6.91	5.62 ± 0.90	3.68 – 6.76	16.38 (Sidwell 1980)
As	0.08 ± 0.05	0.01 – 0.15	0.08 ± 0.04	0.01 – 0.13	0.02 (Sidwell 1980)

4.10 Fatty Acid Results:

Table 29 gives fatty acids and their levels (%) observed in boiled and steamed crawfish samples through 11 days of refrigerated storage. Table 30 has the fatty acid values (%) of boiled and steamed crawfish through six months of frozen storage.

Table 29. Percentages of Fatty Acids Present in Boiled (Top Value) and Steamed (Bottom Value) Crawfish Fat Through 11 Days of Refrigerated (4°C) Storage. Fatty Acids Separated Into Groups: Saturated Fatty Acids (SFA), Mono-unsaturated Fatty Acids (MUFA), and Polyunsaturated Fatty Acids (PUFA).

	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
% SFA Boiled	38.33	33.51	36.20	72.86	63.00	42.78	26.05
% SFA Steamed	41.20	24.93	58.39	56.81	56.94	27.55	16.18
% MUFA Boiled	23.34	25.32	24.43	8.47	7.80	18.16	29.67
% MUFA Steamed	24.69	26.29	9.60	9.80	13.71	41.23	37.95
% PUFA Boiled	38.33	41.17	40.36	18.67	29.20	39.07	44.28
% PUFA Steamed	34.10	48.78	32.01	33.39	29.35	31.22	45.86

Table 30. Percentages of Fatty Acids Present in Boiled (Top Value) and Steamed (Bottom Value) Crawfish Fat Through 6 Months of Frozen Storage (-18°C). Fatty Acids Separated Into Groups: Saturated Fatty Acids (SFA), Mono-unsaturated Fatty Acids (MUFA), and Polyunsaturated Fatty Acids (PUFA).

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
% SFA Boiled	41.04	44.58	36.50	45.83	42.21	36.17
% SFA Steamed	32.95	38.24	33.32	37.15	29.67	35.50
% MUFA Boiled	16.01	22.73	26.51	27.05	29.00	26.19
% MUFA Steamed	27.87	24.98	28.32	29.76	30.39	27.12
% PUFA Boiled	42.95	32.65	36.99	27.11	28.78	37.64
% PUFA Steamed	39.18	36.79	38.36	33.09	39.94	37.38

CHAPTER 5: CONCLUSIONS

Considering all the data that has been presented in this study regarding the quality and shelf life of whole cooked crawfish through refrigerated and frozen storage, there is no overwhelming evidence to conclude that either steaming or boiling produces a superior product. Yields from both cooking methods do not support claims that recovered tail meat yield is far greater in steamed crawfish versus boiled crawfish. Both cooking methods had the same shelf life through refrigerated and frozen storage, three days and six months respectively. These observations suggest that commercial crawfish processors would not benefit by investing in commercial steamers and steaming their crawfish, but should continue boiling their crawfish.

A further study with analyses conducted on each day of storage would allow for a more precise determination of the effect of cooking method on the shelf life of crawfish in terms of their aerobic counts. With either method of cooking crawfish, the refrigerated shelf life is very short for whole cooked crawfish and even four days of refrigerated storage is not sufficient for many marketing channels. A determination of surface moisture versus overall moisture could be revealing and indicative if boiled crawfish were less prone to microbial growth than steamed crawfish. Further research on sensory properties would be advantageous to determine consumer acceptability on the refrigerated and frozen crawfish to compare with the analytical results observed in this study. Also, sensory analysis may reveal a difference between boiling and steaming from a consumer's perspective even though analytical results obtained in this study do not suggest there are any major differences.

REFERENCES

Amr AS, and Rutledge JE 1980. Oxidative Rancidity in Whole-Glazed Frozen Crawfish. Proceedings of the 5th Anuual Tropical & Subtropical Fisheries Technologists Conference April 27-30, 1980 Charleston, SC. p. 214-224.

AOAC. 2005. Official methods of analysis: 18th edition. AOAC International, Gaithersburg, MD.

Barnett, J. 1985. Cajun – can supply meet red-hot demand? Seafood Business Report 4(5): 51.

Bligh EG and Dyer WJ. 1959. A Rapid Method of Total Lipid Extraction and Purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Codex Alimentarius. 2014. http://www.codexalimentarius.org/scientific-basis/jecfa/en/. Accessed March 31, 2015.

Connell JJ. 1964. Fish muscle proteins and some effects on them of processing. In: Shultz, HW and Angelemier AF. Proteins and Their Reactions. AVI Publishing Co., Westport, Ct. pp. 225

Chen G, Xiong YL, Kong B, Newman MC, Thompson KR, Metts LS, and Webster CD. 2007. Microbiological and physiochemical properties of red claw crayfish (*Cherax quadricarinatus)* stored in different package systems at 2°C. J Food Sci 72: 8. E442-E449.

Cox, N.A. and Lovell, R.T. 1973. Identification and characterization of the microflora and spoilage bacteria in freshwater crayfish *Procambarus clarkii.* J. Food Sci 38: 679-681.

Cremades O, Alvarez-Ossorio C, Guttierrez-Gil JF, Parrado J, and Bautista J. 2011. Quality Changes of Cooked Crayfish (*Procambarus Clarkii*) Tails Without Additives During Storage Under Protective Atmospheres. J of Food Process 35: 898-906.

Davis, JT. 1994. Crawfish Production. Aquaculture Technical Series. Cooperative Extension Service, The University of Georgia College of Agriculture and Environmental Sciences. Cooperative State Research Service and Extension Service, US Department of Agriculture Project No. 87-EXCA-3-0836.

Dellenbarger LE, Roberts KR, Kelly SS, and Pawlyk PW. 1986. An analysis of the Louisiana crawfish processing industry and potential market outlets. DAE research report-Department of Agricultural Economics and Agribusiness, Louisiana State University, Louisiana Agricultural Experiment Station (USA).

Ehira S, and Uchiyama H. 1987. Biochemical changes in relation to freshness. In: Kramer DE and Liston J of Seafood Quality Determination. Elsevier, New York. Pp. 185-204.

FDA, 2011. Fish and Fishery Products Hazards and Control Guidance. U.S. Department of Health and Human Services. Food and Drug Administration, Center for Food Safety and Applied Nutrition. pp. 422.

FDA, 2013. Analytical Results from Inorganic Arsenic in Rice and Rice Products Sampling September 2013. United States Food and Drug Administration. Accessed 9/28/2014. http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm319870.htm

Godber, JS, Wang, J, Cole, MT, and Marshall, GA. 1989. Textural Attributes of Mechanically and Cryogenically Frozen Whole Crawfish (Procambarus *clarkii).* J of Food Science 54: 564-566.

Godber JS, Wang J, Moertle, GM, Omoike II, Finerty MW, Grodner RM, and Moody MW. 1986. The effect of residual hepatopancreatic tissue and freezing method on proteolytic activity in crayfish tail meat as related by free amino acids and texture. Abstract No. 394, 46th Annual Meeting of the Institute of Food Technologists, Dallas, TX.

Grodner RM and Novak AF. 1974. Microbiological guidelines for freshwater crayfish (*Procambarus clarkii*). In; Auault JW (Ed) Second International Symposium on Freshwater Crawfish. Louisiana State University, Baton Rouge. pp161-170.

Heitkemper DT, Vela NP, and Stewart KR. 2001. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry J. Anal. At. Specrom 16: 299– 306.

Henckel P, Sivertsvik M, Lunestad BT, Lorentzen G, and Rosens JT. 2007. Characterisation of dominant bacterial population in modified atmosphere packaged farmed halibut (*Hippoglossus hippoglossus)* based on the 16s rDNA-DGGE. Food Micro. 24: 362-371.

Hobbs HH. 1974. A checklist of the North and Middle American crayfishes (Decapoda: Astacidae and Crambaridae). Smith Contrib. Zool: 66, 161.

Horst J. 2010. The Louisiana Seafood Bible: Crawfish. Pelican Publishing Company. Gretna, La.

Horst J, Roberts K. 1993. So You Want To Be A Seafood Dealer, LSU-G-87-002. Louisiana Sea Grant College Program, Communications Office, LSU, Baton Rouge, La 70803-7507.

Huner JV, Payne JF, Bouchard RW, Lorman J, and Magnuson JJ. 1978. Crayfishes in North America (series of articles). Fisheries, 3(6): 2-19.

Hutton MI. 1973. The effects of water velocity, photoperiod, and stubstrate on the survival and growth of subyearling Oregon crayfish (Pacifastacus trowbridgii) Stimpson. Masters Thesis, Fisheries, Oregon State University. pp. 59

ICMSF. 1986. Microogranisms in Foods 2: Sampiling for Microbiological Analysis: Principles and Specific Applications. 2nd Ed. Toronto. University of Toronto Press. p 60-75

Jittinandana S, Kenney PB, and Slider SD. 2003. Cryoprotection affects physiochemical attributes of rainbow trout fillets. J of Food Sci 68: 4, 1208-1214.

Kim HR, Meyers SP, and Godber SJ. 1996. Anionic Trypsins from Crayfish Hepatopancreas: Effects on Protein Degradation of Tail Meat. J of Food Sci: 61, 1. pp. 78, 79.

Lampila LE, Mohr V, and Reid DS. 1985. Scanning electron microscopic study of rockfish preserved at either ambient temperature or by isothermal freeze-fixation. Food Microstructure. 4: 11-16.

Laursen, BG, Leisner JJ, and Dalgaard P. 2006. *Carnobacterium* species: Effect of metabolic activity and interaction with *Brochothrix thermosphacta* on sensory characteristic of modified atmosphere packed shrimp. J. Agric. Food Chem: 54, 3604-3611.

Lemon, DW. 1975. An improved TBA test for rancidity. New Series Circular No. 51. Environment Canada, Fisheries and Marine Service.

Licciardello, JJ. "Freezing." The seafood industry. Springer US, 1990. 205-218.

Louisiana Sea Grant. 1993. Crawfish: Louisiana's Gift. Louisiana Sea Grant College Program, Baton Rouge, LA. Pp 4.

Lovell, Richard. 1968. Development of a Crawfish Processing Industry in Louisiana, a Technical Assistance Project. United States Department of Commerce, Economic Development Administration. pp. 151.

Lovell RT, and Barkate JA. 1969. Incidence and Growth of Some Health-Related Bacteria in Commercial Freshwater Crayfish (Genus Procambarus). J of Food Sci: 34. pp 268-271

Masniyom P, Benjakul S, and Visessanguam W. 2002. Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging. J. Sci. Food Agric: 82, 873-880.

Mason JC. 1963. Life history and production of the crayfish, *Pacifastacus leniusculus trawbridgii* (Stimpson), in a small woodland stream. Masters Thesis, Fisheries, Oregon State University. pp. 204.

Marshall GA, Moody MW, and Hackney CR. 1988. Difference in Color, Texture, and Flavor of Processed Mean from Red Swamp Crawfish (Procambarus clarkii) and White River Crawfish (P. acutus acutus). J of Food Sci. Vol. 53, No. 1. pp. 280-281.

Marshall GA, Moody MW, Hackney CR, and Godber JS. 1987. Effect of Blanch Time on the Development of Mushiness in Ice-Stored Crawfish Meat Packed with Adhering Hepatopancreas. J of Food Sci: 52, 6. p 1504-1505.

McClain RW, and Romaire RP. Crawfish culture: A Louisiana aquaculture success story. World Aquaculture 33:4 pp 31-35, 60-61.

Moertle G, Moody M, and Hackney C. 1985. Processing Time Effects on the Texture of Fresh Packed Crawfish Meat. In: Ward D, Treece G. Proceedings of the 10th Annual Tropical and Subtropical Fisheries Conference of the Americas. pp 119-124.

Moody MW. 1989. Processing of Freshwater Crawfish: A Review. J of Shellfish Research: 8, No.1. pp 293-301.

Moody MW, and Culley D. 1991. Unpublished Data. Evaluation of soft-shell crawfish processing and packaging parameters. Louisiana State University Agricultural Center, Baton Rouge, LA.

Miget RJ. 1991. Microbiology of Crustacean Processing: Shrimp, Crawfish, and Prawns. Ward DR and Hackney CR, editors. Microbiology of Marine Food Products. New York: Van Nostrand Reinhold. p. 65-83.

Nadarajah K, Prinyawiwatkul W, No HK, Harrison WR, and Chen F. 2013. Storage Stability of Minced Meat Extracted from Undersized Crawfish.

NOAA. 2014. Annual Commercial Landing Statistics. http://www.st.nmfs.noaa.gov/pls/webpls/MF_ANNUAL_LANDINGS.RESULTS accessed October 22, 2014.

Ori L, Amacher MC, and Sedberry JE. Jr. 1993. Survey of the total arsenic content in soil in Lousiana. Commun. Soil Sci. Plant Anal 24: 2321–2332.

Penn, GH. 1943. A Study of the Life History of the Louisiana Red-Crawfish, *Cambarus Clarkii* Girard. Ecology Vol. 24 No. 1. pp 1-10.

Pitre, G. 1993. The Crawfish Book. University Press of Mississippi pp 23-25.

Reames, B. 2010. Nutritionalist debunks crawfish myths. LSU AgCenter. 2010. http://text.lsuagcenter.com/news_archive/2010/april/news_you_can_use/Nutritionist-debunks-crawfish-myths.htm. Accessed 9/13/2014.

Reddy MB, and Love M. 1999. The impact of food processing on the nutritional quality of vitamins and minerals." In: Impact of processing on food safety. Springer, US. pp 99-106.

Romaire RP, McClain RW, Shirley MG, and Lutz GC. 2005. Crawfish Aquaculture – Marketing. Louisiana State University Agricultural Center. Southern Regional Aquaculture Center Publication No. 2402.

Ronsivalli LJ, and Charm SE. 1975. Spoilage and Shelf Life Prediction of Refrigerated Fish. MFR Paper 1138. Marine Fisheries Review, Vol. 37, No. 4. pp 32.

Rowland B, Finne G, and Tillman R. A morphological study of muscle proteolysis in the tails of Macrobrachium rosenbergii." Proc. 7th Annual Trop. Subtrop. Fish. Technol. Conf. Amer. Vol. 8. 1982: pp 105-112.

Shenouda, Soliman YK. Theories of protein denaturation during frozen storage of fish flesh. Advances in food research 26 (1980): pp 281-282.

Sidwell VD. 1981. Chemical and Nutritional Composition of Finfishes, Whales, Crustaceans, Mollusks, and Their Products. NOAA Technical Memorandum NMF F/SEC-11. United States Department of Commerce.

Sikorski Z, Olley J, and Kostuch S. 1976 Protein changes in frozen fish. CRC critical reviews in food science and nutrition 8.1 (1976): pp 97.

Szczesniak AS, and Torgeson KW. 1965. Methods of meat texture measurement viewed from the background of factors affecting tenderness. Adv. In Food Res: 14,33.

Treece, GD, Nickelson R, and Finne, G. ****. The Effects of Brine Freezing Crawfish. http://fshn.ifas.ufl.edu/seafood/sst/AnnPdf/10th_173.pdf. Accessed 2/20/2014

Tseng YC, Xiong YL, and Webster CD. 2005. The preservation of the quality of the muscle in frozen Australian red claw crayfish (Cherax quadricarinatus) by pre-storage anti-oxidant dipping treatments. Int. J of Food Sci: 40, 841-848.

USDA. Basic Report: 15146, Crustaceans, crayfish, mixed species, wild, cooked, moist heat. Agricultural Research Service, United States Department of Agriculture. http://ndb.nal.usda.gov/ndb/foods/show/4598?fg=Finfish+and+Shellfish+Products&man=&lfacet=&format=&count=&max=25&offset=125&sort=&qlookup= Accessed: 9/15/2014.

Vynckev W. 1970. Evalutaion of the direct thiobarbituric acid extraction method for determining oxidative rancidity (*Scomber sombrous L).* Seifen-Anstrichmittel 77. p 239-240

Walls J. 2009. Crawfishes of Louisiana. Louisiana State University Press. Baton Rouge. Pp 1-13.

Zeng QZ, Thorarinsdottir KA, and Olafsdotttir G. 2005. Quality changes of shrimp (Pandalus borealis) stored under different cooling conditions. J. of Food Sci: 70, 459-466.

APPENDIX

ANOVA Table for Percent (%) Moisture of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.12160952	0.12160952	0.28	0.5994
TIME	6	19.26630000	3.21105000	7.45	< .0001
TRT * TIME	6	3.59902381	0.59983730	1.39	0.2524

ANOVA Table for Percent (%) Moisture of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.02675238	0.02675238	1.73	0.1993
TIME	6	7.82761429	1.30460238	84.30	< .0001
TRT * TIME	6	5.50094762	0.91682460	59.24	< .0001

ANOVA Table for Percent (%) Ash of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.02031264	0.02031264	3.46	0.0738
TIME	6	0.57392157	0.09565359	16.29	< .0001
TRT * TIME	6	0.03796039	0.00633007	1.08	0.4000

ANOVA Table for Percent (%) Ash of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.10527816	0.10527816	7.65	0.0101
TIME	6	0.15888824	0.02648137	1.92	0.1131
TRT * TIME	6	0.21537843	0.03589641	2.61	0.0399

ANOVA Table for Percent (%) Protein of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	3.66074060	3.66074060	1184.57	< .0001
TIME	6	14.93096814	2.48849469	805.24	< .0001
TRT * TIME	6	2.22044497	0.37007416	119.75	< .0001

ANOVA Table for Percent (%) Protein of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	1.62270548	1.62270548	274.25	< .0001
TIME	6	2.66455891	0.44409315	75.06	< .0001
TRT * TIME	6	2.64025674	0.44004279	74.37	< .0001

ANOVA Table for Percent (%) Fat of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.36571429	0.36571429	21.28	0.0004
TIME	6	2.83354286	0.47225714	27.48	< .0001
TRT * TIME	6	2.22708571	0.37118095	21.60	< .0001

ANOVA Table for Percent (%) Fat of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.31928929	0.31928929	30.13	< .0001
TIME	6	1.11089286	0.18514881	17.47	< .0001
TRT * TIME	6	0.82933571	0.13822262	13.04	< .0001

ANOVA Table for TBARS Values of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.00503810	0.00503810	11.02	0.0025
TIME	6	0.51001429	0.08500238	185.94	< .0001
TRT * TIME	6	0.02486190	0.00414365	9.06	< .0001

ANOVA Table for TBARS Values of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.00053571	0.00053571	2.08	0.1600
TIME	6	0.03928095	0.00654683	25.46	< .0001
TRT * TIME	6	0.02108095	0.00351349	13.66	< .0001

ANOVA Table for Force (g) Values of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	48767128.61	48767128.61	13.38	0.0010
TIME	6	44288230.80	7381371.80	2.02	0.0957
TRT * TIME	6	37577019.45	6262836.57	1.72	0.1537

ANOVA Table for Force (g) Values of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	39283159.1	39283159.1	10.78	0.0028
TIME	6	116930692.2	19488448.7	5.35	0.0009
TRT * TIME	6	26084983.0	4347497.2	1.19	0.3389

ANOVA Table for Work (g/s) Values of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	1279417762	1279417762	12.78	0.0013
TIME	6	1630626290	271771048	2.71	0.0332
TRT * TIME	6	1269583095	211597183	2.11	0.0833

ANOVA Table for Work (g/s) Values of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	1286293442	1286293442	10.46	0.0031
TIME	6	3910216572	651702762	5.30	0.0009
TRT * TIME	6	1645299337	274216556	2.23	0.0697

ANOVA Table for pH Values of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.06880952	0.06880952	41.29	< .0001
TIME	6	0.52285714	0.08714286	52.29	< .0001
TRT * TIME	6	0.26285714	0.04380952	26.29	< .0001

ANOVA Table for pH Values of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	0.0095238	0.00095238	0.50	0.4853
TIME	6	0.90333333	0.15055556	79.04	< .0001
TRT * TIME	6	0.12238095	0.02039683	10.71	< .0001

ANOVA Table for Micro Results (CFU) of Refrigerated Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	1.3580411^{17}	1.3580411^{17}	152.26	< .0001
TIME	6	8.009159^{17}	1.3348598^{17}	149.66	< .0001
TRT * TIME	5	2.733927^{17}	5.467854^{16}	61.30	< .0001

ANOVA Table for Micro Results (CFU) of Frozen Samples

Source	DF	Type III SS	Mean Square	F Value	P > F
TRT	1	68600.000	68600.000	0.72	0.4019
TIME	6	2323085.714	387180.952	4.05	0.0027
TRT * TIME	6	1788000.000	298000.000	3.12	0.0129

ANOVA Table Refrigerated L* Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	17.7539075	17.7639075	0.47	0.4949
TIME	5	540.7804342	108.1560868	2.86	0.0184
TRT * TIME	5	128.1875875	25.6375175	0.68	0.6418

ANOVA Table Refrigerated **a** Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	80.245808	80.245808	2.10	0.1498
TIME	5	1258.580758	251.716152	6.60	<.0001
TRT * TIME	5	416.310378	83.262076	2.18	0.0613

ANOVA Table Refrigerated **b** Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	4.381541	4.381541	0.16	0.6884
TIME	5	2707.117428	541.423486	19.98	<.0001
TRT * TIME	5	242.780394	48.556079	1.79	0.1206

ANOVA Table Frozen L* Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.0770133	0.0770133	0.00	0.9670
TIME	5	374.6985367	74.9397073	1.67	0.1482
TRT * TIME	5	254.9832067	50.9966413	1.14	0.3459

ANOVA Table Frozen **a** Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	18.9369075	18.9369075	0.46	0.4981
TIME	5	358.8595742	71.7719148	1.75	0.1290
TRT * TIME	5	385.0479475	77.0095895	1.88	0.1038

ANOVA Table Aluminum (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	13.6890075	13.6890074	0.63	0.4274
TIME	5	240.3246942	48.0649388	2.23	0.0566
TRT * TIME	5	25.0385875	5.0077175	0.23	0.9477

ANOVA Table Aluminum (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	16.7571416	16.7571416	27.87	< .0001
TIME	12	422.6173873	35.2181156	58.57	< .0001
TRT * TIME	12	116.9341992	9.7445166	16.20	< .0001

ANOVA Table Iron (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	39.4571365	39.4571365	79.51	< .0001
TIME	12	136.6004131	11.3833678	22.94	< .0001
TRT * TIME	12	193.2583553	16.1048629	32.45	< .0001

ANOVA Table Lithium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.06026237	0.06026237	0.69	0.4136
TIME	12	2.44187022	0.20348919	2.33	0.0345
TRT * TIME	12	0.26741047	0.02228421	0.26	0.9917

ANOVA Table Magnesium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	241.6498742	241.6498742	17.15	0.0003
TIME	12	714.0801961	59.5066830	4.22	0.0010
TRT * TIME	12	389.6937773	32.4744814	2.30	0.0364

ANOVA Table Manganese (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00131404	0.00131404	0.96	0.3372
TIME	12	1.67861024	0.13988419	101.78	< .0001
TRT * TIME	12	1.14499012	0.09541584	69.42	< .0001

ANOVA Table Molybenum (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00000734	0.00000734	4.12	0.0526
TIME	12	0.00001578	0.00000132	0.74	0.7019
TRT * TIME	12	0.00001578	0.00000132	0.74	0.7019

ANOVA Table Nickel (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00014526	0.00014526	2.42	0.1317
TIME	12	0.00456599	0.00038050	6.35	< .0001
TRT * TIME	12	0.00171136	0.00014261	2.38	0.0313

ANOVA Table Potassium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	65180.4484	65180.4484	91.89	< .0001
TIME	12	138317.3705	11526.4475	16.25	< .0001
TRT * TIME	12	11308.2015	942.3501	1.33	0.2618

ANOVA Table Silicon (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.0440690	0.0440690	0.06	0.8017
TIME	12	495.5948965	41.2995747	60.34	< .0001
TRT * TIME	12	45.1150290	3.7595857	5.49	< .0001

ANOVA Table Silver (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00008695	0.00008695	1.70	0.2032
TIME	12	0.00132647	0.00006272	1.23	0.3161
TRT * TIME	12	0.00290229	0.00006135	1.20	0.3323

ANOVA Table Sodium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	22433.77090	22433.77090	84.02	< .0001
TIME	12	38826.26709	3235.52226	12.12	< .0001
TRT * TIME	12	10641.23202	886.76933	3.32	< .0001

ANOVA Table Strontium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.23440185	0.23440185	46.95	< .0001
TIME	12	4.75445106	0.39620426	79.35	< .0001
TRT * TIME	12	0.51651297	0.04304275	8.62	< .0001

ANOVA Table Thallium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00056283	0.0056283	0.47	0.4992
TIME	12	0.03553429	00.296119	2.47	0.0260
TRT * TIME	12	0.01385388	0.00115449	0.96	0.5054

ANOVA Table Tin (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00308502	0.00308502	6.42	0.0177
TIME	12	0.01074761	0.00089563	1.86	0.0896
TRT * TIME	12	0.01096491	0.00091374	1.90	0.0830

ANOVA Table Titanium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00823487	0.00823487	8.76	0.0065
TIME	12	0.73853279	0.06154440	65.50	< .0001
TRT * TIME	12	0.29351092	0.02445924	26.03	< .0001

ANOVA Table Antimony (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00014264	0.00014264	0.19	0.6664
TIME	12	0.01391197	0.00115933	1.54	0.1709
TRT * TIME	12	0.00744656	0.00062055	0.83	0.6232

ANOVA Table Arsenic (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00018300	0.00018300	0.08	0.7844
TIME	12	0.03578561	0.00298213	1.24	0.3070
TRT * TIME	12	0.04890964	0.00407580	1.70	0.1245

ANOVA Table Barium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.7705236	0.07705236	6.62	0.0162
TIME	12	5.31916201	0.44326350	38.07	< .0001
TRT * TIME	12	2.68437615	0.22369801	19.21	< .0001

ANOVA Table Beryllium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00000001	0.00000001	0.22	0.6431
TIME	12	0.00017199	0.00001433	440.29	< .0001
TRT * TIME	12	0.00000053	0.00000004	1.37	0.2419

ANOVA Table Boron (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.31403854	0.31403854	2.13	0.1562
TIME	12	3.61531592	0.30127633	2.05	0.0616
TRT * TIME	12	0.64722919	0.05393577	0.37	0.9643

ANOVA Table Cadmium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00000120	0.00000120	0.37	0.5498
TIME	12	0.00286565	0.00023880	73.05	< .0001
TRT * TIME	12	0.00038692	0.00003224	9.86	< .0001

ANOVA Table Chromium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00008825	0.00008825	1.85	0.1858
TIME	12	0.01171496	0.00097625	20.44	< .0001
TRT * TIME	12	0.00764972	0.00063748	13.34	< .0001

ANOVA Table Cobalt (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00001251	0.00001251	0.48	0.4967
TIME	12	0.00875634	0.00072969	27.71	< .0001
TRT * TIME	12	0.0018670	0.00001556	0.59	0.8297

ANOVA Table Copper (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.46879284	0.46879284	7.15	0.0128
TIME	12	25.25060027	2.10421669	32.11	< .0001
TRT * TIME	12	10.72678040	0.89389837	13.64	< .0001

ANOVA Table Vanadium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00004171	0.00004171	2.11	0.1587
TIME	12	0.00341721	0.00028477	14.38	< .0001
TRT * TIME	12	0.00048850	0.00004071	2.06	0.0604

ANOVA Table Zinc (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.10787435	1.10787435	14.17	0.0009
TIME	12	24.29264276	2.02438690	25.89	< .0001
TRT * TIME	12	7.26648042	0.60554003	7.74	< .0001

ANOVA Table Bismuth (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00004328	0.00004328	9.55	0.0047
TIME	12	0.00018589	0.00001549	3.42	0.0042
TRT * TIME	12	0.00019193	0.00001599	3.53	0.0034

ANOVA Table Calcium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	26276.0379	26276.0379	50.81	< .0001
TIME	12	213442.7352	17786.8946	34.39	< .0001
TRT * TIME	12	21170.5855	1764.2155	3.41	0.0043

ANOVA Table Phosphorous (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1231.7256	1231.7256	1.07	0.3113
TIME	12	142321.7854	11860.1488	10.27	< .0001
TRT * TIME	12	20036.9522	1669.7460	1.45	0.2084

ANOVA Table Selenium (ppm) Values

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00155858	0.00155858	1.01	0.3241
TIME	12	0.05375713	0.00447976	2.90	0.0111
TRT * TIME	12	0.00743062	0.00061922	0.40	0.9500

Table 27. Percentages of Fatty Acids Present in Boiled (Top Value) and Steamed (Bottom Value) Crawfish Fat Through 11 Days of Refrigerated (4°C). Fatty Acids Separated Into Groups: Saturated Fatty Acids (SFA), Mono-unsaturated Fatty Acids (MUFA), and Polyunsaturated Fatty Acids (PUFA).

	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
SFA %							
C4:0	0.01 0.01	0.01 0.13	0.01 0.00	0.00 0.00	0.01 0.00	0.00 0.00	2.50 0.00
C6:0	0.83 1.01	0.78 0.29	1.11 1.96	2.50 1.72	5.42 1.26	1.00 0.41	0.57 0.20
C8:0	0.13 0.16	0.11 0.05	0.17 0.00	0.00 0.00	0.01 0.00	5.06 2.27	3.15 1.13
C10:0	0.45 0.33	0.29 0.01	0.34 0.62	0.65 0.45	1.32 0.38	2.41 1.08	1.51 0.55
C11:0	0.52 0.61	0.48 0.17	0.67 0.25	0.31 0.21	0.66 0.15	0.57 0.26	0.36 0.13
C12:0	0.26 0.26	0.23 0.08	0.27 1.20	1.50 1.03	3.26 0.76	0.49 0.21	0.30 0.13
C13:0	0.40 0.48	0.37 0.14	0.51 1.03	1.29 0.90	2.83 0.66	0.65 0.29	0.41 0.17
C14:0	1.58 1.68	1.37 0.76	1.73 1.75	2.07 1.46	4.28 1.16	1.55 0.86	1.16 1.01
C15:0	0.68 0.66	0.59 0.47	0.60 0.80	0.87 0.59	1.07 0.64	0.52 0.37	0.00 1.19
C16:0	19.09 20.74	15.67 15.20	14.58 13.01	26.32 15.95	8.98 24.88	7.74 9.12	6.09 3.43

C17:0	1.06 1.12	0.88 0.41	1.26 1.40	1.55 2.52	1.24 0.44	1.40 0.87	1.11 0.82
C18:0	10.55 10.88	10.17 6.21	11.35 12.33	8.02 17.48	8.13 11.81	4.60 4.24	3.63 3.53
C20:0	1.11 1.34	1.06 0.42	1.44 2.24	0.17 1.95	6.14 1.42	4.02 1.82	2.50 1.00
C21:0	1.58 1.85	1.42 0.57	1.96 2.38	3.02 2.07	6.52 1.51	3.80 1.71	2.48 0.86
C22:0	0.07 0.08	0.06 0.02	0.08 3.21	4.07 2.81	8.83 2.04	8.87 3.99	0.30 2.01
C23:0	0.01 0.01	0.01 0.00	0.01 1.65	2.02 1.36	4.30 0.50	0.09 0.02	0.07 0.02
C24:0	0.01 0.01	0.01 0.00	0.01 14.47	18.50 6.31	0.01 9.33	0.00 0.00	0.00 0.00
SFA total	**38.33** **41.20**	**33.51** **24.93**	**36.20** **58.39**	**72.86** **56.81**	**63.00** **56.94**	**42.78** **27.55**	**26.05** **16.18**
MUFA %							
C14:1	0.01 0.01	0.01 0.00	0.01 0.50	0.63 0.43	0.01 0.32	0.47 0.21	0.30 0.12
C15:1	0.01 0.86	0.65 0.25	0.50 0.02	0.03 0.02	0.07 0.01	0.49 0.22	0.80 0.13
C16:1	3.56 3.91	3.20 4.05	2.34 7.41	6.44 8.14	4.02 11.95	2.87 4.23	1.97 0.40
C17:1	1.07 1.23	0.65 0.48	0.50 0.14	0.03 0.12	0.07 0.20	0.49 0.57	0.80 0.69
C18:1	14.60	16.48	14.01	0.35	1.59	8.42	16.27

	13.88	19.88	0.61	0.26	0.38	22.86	27.07
C18:1 (trans)	0.01 0.01	0.01 0.00	0.01 0.00	0.00 0.00	0.01 0.00	3.09 11.50	8.27 8.60
C20:1	3.98 4.65	3.77 1.59	5.04 0.73	0.84 0.66	1.75 0.45	1.73 0.95	1.53 0.78
C22:1	0.01 0.01	0.01 0.00	0.01 0.00	0.00 0.00	0.01 0.40	0.00 0.02	0.04 0.00
C24:1	0.09 0.13	0.14 0.04	0.23 0.18	0.00 0.17	0.01 0.00	0.00 0.67	0.00 0.17
MUFA % total	**23.34** **24.69**	**25.32** **26.29**	**24.43** **9.60**	**8.47** **9.80**	**7.80** **13.71**	**18.16** **41.23**	**29.67** **37.95**
PUFA %							
C18:2	18.76 10.20	18.56 36.53	13.30 21.24	8.92 14.42	12.53 12.17	11.24 13.88	21.94 22.27
C18:2 (trans)	0.01 0.01	0.01 0.00	0.01 0.00	0.00 0.00	0.01 0.00	0.00 0.00	0.00 0.00
C18:3 (6,9,12)	1.56 3.74	2.93 1.10	4.09 1.18	1.47 1.05	1.64 0.76	2.22 0.99	1.39 0.53
C18:3 (9,12,15)	0.68 1.09	1.60 1.69	0.95 4.03	2.65 3.84	3.93 2.81	2.83 2.17	2.24 6.89
C20:2 (11,14)	1.00 1.04	0.86 0.45	1.16 0.35	0.29 0.55	0.01 0.15	1.42 0.93	0.91 0.88
C20:2 (13,16)	1.74 2.12	1.63 0.61	2.31 0.00	0.00 0.00	1.50 0.00	1.96 0.89	2.48 0.50
C20:3 (8,11,14)	0.64 0.68	0.58 0.33	0.93 1.43	1.81 1.29	3.84 0.92	2.75 1.27	1.77 0.71

C20:3 (11,14,17)	1.80 2.29	1.78 0.70	2.56 0.42	1.02 0.76	1.13 0.53	7.42 3.55	4.90 2.25
C20:4	7.99 8.62	8.26 3.80	9.69 1.83	2.33 2.02	4.59 1.44	4.80 2.78	3.92 4.20
C20:5	4.15 4.30	4.96 2.63	5.36 0.67	0.17 7.94	0.01 6.46	2.94 2.95	2.65 5.64
C22:6	0.01 0.01	0.01 0.93	0.01 0.85	0.00 1.51	0.01 4.11	1.47 1.81	2.06 2.00
PUFA total	**38.33** **34.10**	**41.17** **48.78**	**40.36** **32.01**	**18.67** **33.39**	**29.20** **29.35**	**39.07** **31.22**	**44.28** **45.86**

Table 29. Percentages of Fatty Acids Present in Boiled (Top Value) and Steamed (Bottom Value) Crawfish Fat Through 6 Months of Frozen Storage (-18°C). Fatty Acids Separated Into Groups: Saturated Fatty Acids (SFA), Mono-unsaturated Fatty Acids (MUFA), and Polyunsaturated Fatty Acids (PUFA).

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
SFA %						
C4:0	0.00 0.01	0.00 0.00	0.00 0.00	0.26 0.00	0.00 0.15	0.46 0.00
C6:0	0.91 2.54	4.89 3.81	3.48 2.68	1.76 1.17	1.65 0.94	1.49 1.20
C8:0	5.10 2.23	4.28 3.33	3.03 2.33	2.72 1.82	2.56 1.46	2.31 1.85
C10:0	2.41 0.37	0.68 0.61	0.50 0.36	0.38 0.31	0.34 0.23	0.37 0.27
C11:0	0.57 0.27	0.52 0.41	0.37 0.28	1.10 0.74	1.04 0.59	0.94 0.75
C12:0	0.46 0.64	1.24 0.96	0.89 0.68	2.13 1.43	2.00 1.14	1.82 1.46
C13:0	0.65 0.38	0.73 0.57	0.60 0.40	0.44 0.30	0.42 0.24	0.38 0.31
C14:0	1.49 1.23	1.96 1.59	1.46 1.28	1.71 1.34	1.67 1.22	1.62 1.62
C15:0	0.39 0.36	0.28 0.27	0.27 0.32	1.32 1.00	1.25 0.99	1.21 1.21
C16:0	10.47 10.88	9.08 9.05	9.25 8.40	8.44 11.36	7.79 8.36	7.06 11.98

C17:0	1.62 1.03	1.52 1.25	1.17 0.93	1.43 1.08	1.38 1.20	1.39 1.21
C18:0	4.65 5.53	5.58 5.33	5.63 4.86	4.22 6.26	5.00 6.16	5.17 5.16
C20:0	4.05 3.13	5.95 4.65	4.23 3.27	3.69 2.48	3.48 2.00	3.16 2.54
C21:0	3.83 1.16	2.10 1.68	1.51 1.24	1.49 1.05	1.45 0.87	1.33 1.10
C22:0	4.36 1.96	3.46 2.78	2.53 2.11	1.35 1.05	1.32 0.93	1.30 1.03
C23:0	0.08 1.21	2.31 1.93	1.58 1.30	13.41 5.77	10.87 3.17	6.18 3.78
C24:0	0.00 0.00	0.00 0.00	0.00 2.86	0.00 0.00	0.00 0.00	0.00 0.00
SFA total	**41.04** **32.95**	**44.58** **38.24**	**36.50** **33.32**	**45.83** **37.15**	**42.21** **29.67**	**36.17** **35.50**
MUFA %						
C14:1	0.47 0.00	0.00 0.00	0.00 0.00	0.66 0.44	0.63 0.36	0.56 0.46
C15:1	0.49 0.02	0.35 0.30	0.52 0.40	1.32 0.89	1.26 0.73	1.13 0.93
C16:1	2.69 3.30	2.89 3.15	3.25 3.25	2.59 3.93	3.71 3.90	2.97 5.50
C17:1	1.09 0.21	0.00 0.08	0.14 0.20	0.33 0.29	0.48 0.69	0.39 0.50
C18:1	7.06	9.43	15.25	17.03	18.66	17.36

	18.71	13.55	19.04	21.22	21.96	16.62
C18:1 (trans)	2.31 0.33	0.00 0.00	0.00 0.00	0.29 0.00	0.00 0.16	0.00 0.00
C20:1	1.83 4.46	7.91 6.23	5.77 4.57	4.16 2.61	3.68 2.31	3.31 2.70
C22:1	0.07 0.27	1.05 0.82	0.75 0.27	0.18 0.07	0.13 0.03	0.07 0.10
C24:1	0.00 0.58	1.09 0.85	0.82 0.60	0.48 0.31	0.46 0.25	0.39 0.31
MUFA % total	**16.01** **27.87**	**22.73** **24.98**	**26.51** **28.32**	**27.05** **29.76**	**29.00** **30.39**	**26.19** **27.12**
PUFA %						
C18:2	14.59 21.87	10.82 15.60	15.93 19.77	15.01 19.46	17.89 21.07	22.61 18.71
C18:2 (trans)	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
C18:3 (6,9,12)	2.24 0.43	0.53 0.47	0.45 0.39	0.17 0.22	0.21 0.34	0.36 0.31
C18:3 (9,12,15)	2.92 7.33	9.93 9.29	8.88 7.64	4.65 4.49	4.63 7.27	5.21 6.83
C20:2 (11,14)	1.52 0.52	0.00 0.11	0.19 0.41	0.00 1.85	0.00 1.50	1.06 1.93
C20:2 (13,16)	4.01 0.03	0.05 0.03	0.04 0.04	0.01 0.00	0.00 0.00	0.00 0.00
C20:3 (8,11,14)	2.79 0.75	1.18 0.99	0.91 0.81	1.75 1.22	1.67 1.06	1.51 1.30

C20:3 (11,14,17)	7.48 0.01	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.68	0.00 0.02
C20:4	4.81 2.67	3.61 3.29	3.50 3.14	3.02 2.73	2.58 3.00	2.31 2.53
C20:5	2.58 3.42	4.75 4.32	4.22 3.78	2.49 3.11	1.81 4.00	3.11 3.44
C22:6	0.00 2.15	1.81 2.67	2.87 2.37	0.00 0.00	0.00 1.01	1.45 2.31
PUFA total	**42.95** **39.18**	**32.65** **36.79**	**36.99** **38.36**	**27.11** **33.09**	**28.78** **39.94**	**37.64** **37.38**

Total Minerals Breakdown:

Aluminum	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	7.90 ± 0.01 IJKL	13.78 ± 0.11 B	11.45 ± 0.50 CD	9.93 ± 1.57 DEFGH	9.50 ± 0.28 FGHI	11.02 ± 0.21 DEF	12.62 ± 0.94 BC	5.17 ± 0.40 NO	7.29 ± 0.77 KLM	4.72 ± 0.43 NO	11.38 ± 0.01 CDE	7.47 ± 0.80 KLM	13.52 ± 0.41 B
Steamed	6.18 ± 0.23 MN	6.79 ± 0.99 LM	16.83 ± 1.09 A	8.48 ± 0.97 HIJK	8.34 ± 0.73 HIJKL	13.63 ± 0.09 B	10.61 ± 1.94 DEFG	4.70 ± 1.14 NO	4.39 ± 0.92 O	4.01 ± 0.06 O	7.86 ± 0.67 JKL	9.32 ± 0.12 GHIJ	9.85 ± 0.31 EFGH

Iron	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	15.43 ± 0.29 CDE	18.61 ± 0.73 A	11.37 ± 0.61 JK	14.74 ± 2.77 EFG	14.99 ± 0.13 DEFG	16.80 ± 0.40 BC	16.36 ± 0.56 BCD	12.42 ± 0.27 HIJ	10.69 ± 0.45 K	10.32 ± 0.48 K	15.95 ± 0.46 CDE	13.71 ± 0.25 GHI	17.70 ± 0.71 AB
Steamed	11.42 ± 0.03 JK	11.42 ± 0.02 JK	13.87 ± 0.38 FGH	6.53 ± 0.19 L	12.53 ± 0.70 HIJ	15.25 ± 0.56 DEF	11.75 ± 0.16 JK	12.68 ± 0.33 HIJ	16.20 ± 0.17 CD	11.71 ± 0.37 JK	12.35 ± 0.95 IJ	16.22 ± 0.40 CD	14.53 ± 0.35 EFG

Lithium	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	0.59 ± 0.09 ABC	0.71 ± 0.38 ABC	0.30 ± 0.18 ABC	0.15 ± 0.06 C	0.19 ± 0.01 C	0.26 ± 0.12 BC	0.20 ± 0.03 C	0.70 ± 0.15 ABC	0.68 ± 0.30 ABC	0.29 ± 0.05 ABC	0.57 ± 0.15 ABC	0.44 ± 0.18 ABC	0.23 ± 0.01 BC
Steamed	0.88 ± 0.91 A	0.73 ± 0.37 ABC	0.28 ± 0.03 ABC	0.15 ± 0.02 C	0.19 ± 0.02 C	0.15 ± 0.05 C	0.20 ± 0.02 BC	0.68 ± 0.43 ABC	0.81 ± 0.52 AB	0.55 ± 0.31 ABC	0.49 ± 0.21 ABC	0.47 ± 0.02 ABC	0.60 ± 0.58 ABC

Magnesium	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	88.77 ± 0.86 ABCD	83.08 ± 2.22 BCDEFGHI	80.01 ± 0.28 FGHI	89.68 ± 14.46 ABC	82.02 ± 0.38 CDEFGHI	79.93 ± 1.04 FGHI	90.36 ± 0.41 AB	89.98 ± 1.39 AB	86.47 ± 0.93 ABCDEFG	92.95 ± 0.28 A	78.02 ± 11.10 HIJ	89.80 ± 0.90 AB	87.97 ± 0.72 ABCDE
Steamed	87.64 ± 2.07 ABCDEF	80.25 ± 2.32 EFGHI	76.18 ± 1.13 IJ	70.97 ± 0.82 J	79.74 ± 0.94 GHI	77.87 ± 0.91 HIJ	81.37 ± 0.92 DEFGHI	84.12 ± 1.08 BCDEFGH	83.16 ± 1.56 BCDEFGHI	85.97 ± 0.23 ABCDEFG	84.53 ± 0.45 BCDEGH	84.40 ± 2.15 BCDEFGH	86.73 ± 0.63 ABCDEFG

Manganese	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	1.22 ± 0.00 BCD	1.06 ± 0.04 FGH	0.41 ± 0.03 K	1.22 ± 0.08 ABC	1.09 ± 0.03 EFG	1.00 ± 0.02 HI	0.95 ± 0.10 I	1.21 ± 0.02 BCD	0.94 ± 0.03 I	0.78 ± 0.00 J	1.15 ± 0.05 CDE	1.25 ± 0.04 AB	1.29 ± 0.02 A
Steamed	1.22 ± 0.00 ABC	1.05 ± 0.03 GH	0.78 ± 0.02 J	0.36 ± 0.00 K	1.25 ± 0.08 AB	1.12 ± 0.04 EFG	1.13 ± 0.01 EF	1.14 ± 0.00 DE	1.05 ± 0.00 GH	1.15 ± 0.01 CDE	1.06 ± 0.02 FGH	1.29 ± 0.02 AB	1.11 ± 0.01 EFG

Molybdenum	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B
Steamed	0.04 ± 0.00 AB	0.04 ± 0.00 B	0.04 ± 0.00 AB	0.04 ± 0.00 B	0.04 ± 0.00 A	0.04 ± 0.00 AB	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 AB	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B	0.04 ± 0.00 B

Nickel	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	0.03 ± 0.00 BCDEFG	0.03 ± 0.00 DEFGHI	0.02 ± 0.00 HIJ	0.03 ± 0.02 CDEFG	0.04 ± 0.00 ABCDEF	0.03 ± 0.00 DEFGHIJ	0.05 ± 0.02 A	0.02 ± 0.00 FGHIJ	0.03 ± 0.00 DEFGH	0.03 ± 0.01 DEFGH	0.01 ± 0.00 J	0.02 ± 0.00 GHIJ	0.02 ± 0.01 DEFGHIJ
Steamed	0.03 ± 0.01 CDEF	0.04 ± 0.01 ABCD	0.03 ± 0.01 DEFGHIJ	0.02 ± 0.01 GHIJ	0.05 ± 0.01 ABC	0.05 ± 0.01 AB	0.04 ± 0.00 ABCD	0.04 ± 0.00 ABCD	0.04 ± 0.00 ABCDE	0.02 ± 0.02 EFGHIJ	0.01 ± 0.00 J	0.02 ± 0.01 DEFGHIJ	0.01 ± 0.00 IJ

Potassium	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	641.46 ± 9.37 CDEFG	573.75 ± 24.47 HIJK	549.98 ± 13.85 JKL	590.97 ± 94.50 GHIJ	543.74 ± 16.14 JKLM	503.94 ± 9.96 LM	494.79 ± 5.23 M	627.23 ± 19.49 DEFGH	569.65 ± 6.63 IJK	641.33 ± 8.05 CDEFG	586.03 ± 11.68 HIJK	571.27 ± 16.61 IJK	598.06 ± 6.94 FGHIJ
Steamed	729.43 ± 24.46 AB	678.76 ± 48.77 BCD	647.46 ± 5.35 CDEF	614.65 ± 2.70 EFGHI	571.09 ± 13.53 IJK	544.17 ± 14.60 JKLM	535.89 ± 2.36 KLM	682.80 ± 12.48 BC	671.90 ± 36.63 CD	738.37 ± 20.59 A	680.65 ± 29.00 BCD	664.18 ± 2.84 CDE	653.35 ± 33.37 CDE

Silicon	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	13.03 ± 0.79 LM	16.45 ± 0.07 GHIJ	15.43 ± 0.42 IJK	15.33 ± 0.49 IJK	17.36 ± 0.07 EFGH	17.42 ± 0.15 EFGH	18.60 ± 1.24 CDEF	11.59 ± 0.39 MNO	13.04 ± 0.17 LM	11.18 ± 0.20 NO	17.33 ± 0.22 EFGH	16.92 ± 1.60 FGHI	21.30 ± 1.01 AB
Steamed	12.07 ± 0.53 MN	14.15 ± 0.34 KL	18.65 ± 0.06 CDE	14.94 ± 0.23 JK	17.29 ± 0.51 EFGH	21.58 ± 2.14 A	19.34 ± 2.21 CD	11.41 ± 0.13 MNO	10.88 ± 0.48 NO	10.22 ± 0.17 O	16.05 ± 0.78 HIJ	17.89 ± 0.39 DEFG	19.77 ± 0.38 BC

Silver	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B
Steamed	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.01 ± 0.00 B	0.04 ± 0.04 A	0.01 ± 0.00 B	0.01 ± 0.00 B

Sodium	Day0	Day1	Day3	Day5	Day7	Day9	Day11	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	356.15 ± 3.93 DEFG	353.42 ± 16.29 DEFG	334.79 ± 0.45 FGH	362.76 ± 63.74 DEF	323.23 ± 0.85 GHI	308.69 ± 12.09 HI	300.21 ± 4.71 I	364.26 ± 0.18 DEF	374.20 ± 10.54 CDE	342.09 ± 2.92 EFGH	332.03 ± 6.86 FGHI	346.80 ± 1.38 EFG	347.06 ± 5.03 EFG

Steamed	405.25 ± 35.36 ABC	411.84 ± 13.00 AB	363.38 ± 1.97 DEF	335.38 ± 2.89 FGH	337.80 ± 5.06 FGH	330.74 ± 4.63 FGHI	325.93 ± 5.23 GHI	430.03 ± 2.68 A	431.24 ± 14.03 A	404.69 ± 5.59 ABC	425.03 ± 0.94 A	385.89 ± 4.42 BCD	398.48 ± 21.77 ABC

Strontium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.94 ± 0.01 OP	1.23 ± 0.05 IJKL	1.25 ± 0.04 HIJK	1.94 ± 0.33 B	1.44 ± 0.02 FG	1.69 ± 0.05 D	1.87 ± 0.01 BC	1.16 ± 0.04 JKLM	1.43 ± 0.07 FG	1.38 ± 0.00 GH	1.49 ± 0.03 FG	1.35 ± 0.01 GHI	1.27 ± 0.01 HIJ
Steamed	0.89 ± 0.01 P	1.09 ± 0.02 LMN	1.11 ± 0.01 KLMN	1.65 ± 0.04 DE	1.53 ± 0.03 EF	1.76 ± 0.00 CD	2.16 ± 0.00 A	1.05 ± 0.01 MNO	0.97 ± 0.02 NOP	1.23 ± 0.00 IJKL	1.11 ± 0.05 KLMN	1.01 ± 0.01 NOP	1.11 ± 0.03 KLMN

Thallium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.08 ± 0.02 ABCDEF	0.08 ± 0.03 ABCDE	0.10 ± 0.04 ABCD	0.09 ± 0.01 ABCDE	0.11 ± 0.01 ABC	0.07 ± 0.08 ABCDEF	0.12 ± 0.02 AB	0.07 ± 0.05 ABCDEF	0.06 ± 0.05 ABCDEF	0.01 ± 0.00 F	0.03 ± 0.03 EF	0.04 ± 0.02 CDEF	0.03 ± 0.01 EF
Steamed	0.09 ± 0.03 ABCDE	0.04 ± 0.02 CDEF	0.05 ± 0.03 BCDEF	0.13 ± 0.09 A	0.10 ± 0.03 ABCD	0.11 ± 0.02 ABC	0.09 ± 0.02 ABCDE	0.06 ± 0.03 ABCDEF	0.07 ± 0.02 ABCDEF	0.03 ± 0.03 EF	0.03 ± 0.03 DEF	0.08 ± 0.02 ABCDEF	0.08 ± 0.01 ABCDEF

Tin	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.01 ± 0.00 D	0.03 ± 0.03 BCD	0.02 ± 0.01 CD	0.05 ± 0.03 BCD	0.04 ± 0.02 BCD	0.04 ± 0.04 BCD	0.01 ± 0.00 D	0.03 ± 0.01 BCD	0.06 ± 0.01 AB	0.06 ± 0.01 ABC	0.01 ± 0.00 D	0.03 ± 0.02 BCD	0.09 ± 0.06 A
Steamed	0.05 ± 0.01 ABCD	0.01 ± 0.00 D	0.01 ± 0.00 D	0.01 ± 0.00 D	0.05 ± 0.05 ABCD	0.01 ± 0.00 D	0.01 ± 0.00 D	0.02 ± 0.01 BCD	0.01 ± 0.00 D	0.05 ± 0.03 BCD	0.01 ± 0.00 D	0.01 ± 0.00 D	0.01 ± 0.01 D

Titanium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.55 ± 0.03 B	0.29 ± 0.01 FG	0.16 ± 0.00 JK	0.18 ± 0.01 JK	0.20 ± 0.01 IJK	0.27 ± 0.01 FGH	0.24 ± 0.04 GHI	0.36 ± 0.01 CDE	0.73 ± 0.11 A	0.08 ± 0.00 L	0.28 ± 0.01 FG	0.21 ± 0.04 HIJ	0.37 ± 0.03 CD
Steamed	0.42 ± 0.05 C	0.17 ± 0.01 JK	0.32 ± 0.01 DEF	0.19 ± 0.00 IJK	0.19 ± 0.01 IJK	0.29 ± 0.03 FG	0.25 ± 0.02 GHI	0.56 ± 0.01 B	0.32 ± 0.05 DEF	0.15 ± 0.01 K	0.20 ± 0.01 IJK	0.30 ± 0.01 EFG	0.25 ± 0.01 GHI

Antimony	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.04 ± 0.01 ABCD	0.07 ± 0.03 ABC	0.03 ± 0.01 BCD	0.03 ± 0.03 BCD	0.02 ± 0.01 CD	0.05 ± 0.02 ABCD	0.07 ± 0.01 ABC	0.08 ± 0.04 AB	0.02 ± 0.01 BCD	0.07 ± 0.06 ABCD	0.02 ± 0.01 BCD	0.08 ± 0.00 AB	0.05 ± 0.06 ABCD
Steamed	0.10 ± 0.02 A	0.05 ± 0.00 ABCD	0.04 ± 0.03 BCD	0.04 ± 0.04 BCD	0.03 ± 0.03 BCD	0.03 ± 0.01 BCD	0.04 ± 0.02 BCD	0.05 ± 0.02 ABCD	0.06 ± 0.02 ABCD	0.05 ± 0.00 ABCD	0.01 ± 0.00 D	0.07 ± 0.03 ABC	0.04 ± 0.04 ABCD

Arsenic	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.06 ± 0.07 ABCDEF	0.11 ± 0.07 ABCDE	0.15 ± 0.11 A	0.01 ± 0.00 F	0.02 ± 0.01 EF	0.09 ± 0.05 ABCDEF	0.12 ± 0.03 ABCD	0.06 ± 0.03 ABCDEF	0.09 ± 0.00 ABCDEF	0.13 ± 0.02 AB	0.08 ± 0.10 ABCDEF	0.03 ± 0.02 DEF	0.13 ± 0.07 ABC
Steamed	0.04 ± 0.03 BCDEF	0.03 ± 0.03 CDEF	0.11 ± 0.05 ABCDEF	0.13 ± 0.01 ABCD	0.13 ± 0.13 ABCD	0.09 ± 0.01 ABCDEF	0.07 ± 0.05 ABCDEF	0.06 ± 0.04 ABCDEF	0.13 ± 0.02 AB	0.07 ± 0.07 ABCDEF	0.01 ± 0.00 F	0.07 ± 0.01 ABCDEF	0.10 ± 0.08 ABCDEF

Barium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.78 ± 0.02 G	1.16 ± 0.12 E	1.19 ± 0.01 E	1.96 ± 0.32 BC	1.19 ± 0.07 E	1.46 ± 0.08 D	1.45 ± 0.03 D	EF	2.04 ± 0.20 BC	1.53 ± 0.15 D	1.87 ± 0.04 C	1.45 ± 0.12 D	1.47 ± 0.05 D
Steamed	0.86 ± 0.06 FG	1.06 ± 0.05 EF	1.19 ± 0.04 E	2.12 ± 0.15 B	1.53 ± 0.03 D	1.44 ± 0.14 D	2.41 ± 0.03 A	1.06 ± 0.05 EF	1.05 ± 0.04 EF	1.45 ± 0.01 D	1.15 ± 0.12 E	1.13 ± 0.01 E	1.16 ± 0.15 E

Beryllium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.01 ± 0.00 EF	0.01 ± 0.00 DEF	0.01 ± 0.00 CDEF	0.01 ± 0.00 EF	0.01 ± 0.00 F	0.01 ± 0.00 DEF	0.02 ± 0.00 C	0.01 ± 0.00 DEF	0.01 ± 0.00 DEF	0.02 ± 0.00 A	0.01 ± 0.00 G	0.02 ± 0.00 B	0.02 ± 0.00 B
Steamed	0.01 ± 0.00 DEF	0.01 ± 0.00 EF	0.02 ± 0.00 CDE	0.00 ± 0.00 DEF	0.02 ± 0.00 CDE	0.02 ± 0.00 CD	0.02 ± 0.00 CDEF	0.01 ± 0.00 EF	0.01 ± 0.00 F	0.02 ± 0.00 A	0.01 ± 0.00 G	0.02 ± 0.00 B	0.02 ± 0.00 B

Boron	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	4.62 ± 0.74 ABC	4.31 ± 0.27 ABCD	4.67 ± 0.35 AB	4.33 ± 0.12 ABCD	4.38 ± 0.16 ABCD	4.77 ± 0.34 AB	4.23 ± 0.56 ABCD	4.00 ± 0.56 BCD	4.00 ± 0.37 BCD	3.73 ± 0.36 D	4.23 ± 0.08 ABCD	4.43 ± 0.19 ABCD	3.84 ± 0.09 CD
Steamed	4.85 ± 0.20 A	4.77 ± 0.10 AB	4.68 ± 0.03 AB	4.28 ± 0.37 ABCD	4.55 ± 0.05 ABC	4.33 ± 0.59 ABCD	4.39 ± 0.52 ABCD	4.24 ± 0.65 ABCD	4.14 ± 0.06 ABCD	3.84 ± 0.28 CD	4.56 ± 0.45 ABC	4.61 ± 0.12 ABC	4.31 ± 0.67 ABCD

Cadmium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.04 ± 0.00 B	0.04 ± 0.00 B	0.03 ± 0.00 DE	0.03 ± 0.00 EFG	0.02 ± 0.00 GHI	0.02 ± 0.00 JKL	0.02 ± 0.00 KLM	0.03 ± 0.00 D	0.03 ± 0.00 DE	0.02 ± 0.00 FG	0.01 ± 0.00 N	0.02 ± 0.00 IJKL	0.02 ± 0.00 KLM
Steamed	0.03 ± 0.00 BC	0.03 ± 0.00 CD	0.03 ± 0.00 DEF	0.02 ± 0.00 M	0.02 ± 0.00 HIJK	0.02 ± 0.00 KLM	0.02 ± 0.00 LM	0.04 ± 0.00 B	0.04 ± 0.00 A	0.03 ± 0.00 EFG	0.01 ± 0.00 N	0.02 ± 0.00 FGH	0.02 ± 0.00 GHIJ

Chromium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.02 ± 0.01 FGHIJ	0.02 ± 0.00 GHIJ	0.01 ± 0.00 IJ	0.03 ± 0.00 DEFGHI	0.09 ± 0.01 A	0.02 ± 0.01 FGHIJ	0.04 ± 0.02 BCD	0.02 ± 0.01 GHIJ	0.02 ± 0.00 FGHIJ	0.01 ± 0.00 J	0.01 ± 0.00 J	0.05 ± 0.00 B	0.03 ± 0.00 CDEFG

Steamed	0.02 ± 0.00 FGHIJ	0.04 ± 0.00 BCDEF	0.04 ± 0.01 BCDE	0.02 ± 0.00 HIJ	0.05 ± 0.00 BC	0.03 ± 0.00 EFGHI	0.04 ± 0.02 BCDE	0.02 ± 0.00 FGHIJ	0.09 ± 0.01 A	0.01 ± 0.00 IJ	0.01 ± 0.00 J	0.02 ± 0.00 GHIJ	0.03 ± 0.00 DEFGH
Cobalt	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.03 ± 0.02 B	0.01 ± 0.00 E	0.01 ± 0.00 E	0.02 ± 0.00 CD	0.01 ± 0.00 E	0.01 ± 0.00 DE	0.02 ± 0.01 DE	0.06 ± 0.00 A	0.02 ± 0.00 DE	0.01 ± 0.01 DE	0.01 ± 0.00 E	0.01 ± 0.00 E	0.01 ± 0.00 E
Steamed	0.03 ± 0.00 BC	0.01 ± 0.00 E	0.01 ± 0.00 DE	0.01 ± 0.00 E	0.01 ± 0.00 E	0.01 ± 0.01 DE	0.01 ± 0.00 DE	0.06 ± 0.01 A	0.02 ± 0.00 DE	0.01 ± 0.00 DE	0.01 ± 0.00 E	0.01 ± 0.00 E	0.01 ± 0.00 E
Copper	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	4.47 ± 0.01 A	2.34 ± 0.06 FGHIJ	1.48 ± 0.09 LM	3.48 ± 0.53 BCD	2.98 ± 0.17 DE	2.52 ± 0.07 EFGH	2.53 ± 0.23 EFGH	3.37 ± 0.50 CD	2.49 ± 0.16 EFGH	1.86 ± 0.09 JKL	2.03 ± 0.11 HIJK	2.22 ± 0.18 FGHIJ	1.96 ± 0.15 IJKL
Steamed	3.51 ± 0.31 BCD	1.96 ± 0.02 IJKL	1.32 ± 0.13 M	0.73 ± 0.13 N	2.57 ± 0.15 EFG	2.16 ± 0.29 GHIJ	2.74 ± 0.11 EF	3.98 ± 0.81 AB	3.61 ± 0.09 BC	2.41 ± 0.13 FGHI	1.63 ± 0.17 KLM	2.51 ± 0.08 EFGH	2.12 ± 0.05 GHIJK
Calcium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	333.74 ± 6.87 IJKL	394.13 ± 18.46 EFGH	389.33 ± 3.16 EFGH	531.22 ± 108.11 AB	431.59 ± 3.51 DE	465.87 ± 16.11 CD	545.91 ± 2.20 A	348.10 ± 3.88 HIJK	357.51 ± 0.35 HIJ	466.88 ± 6.88 CD	414.99 ± 9.09 EFG	359.61 ± 7.24 HIJ	371.19 ± 3.14 GHI
Steamed	297.53 ± 9.65 L	323.18 ± 9.73 JKL	323.45 ± 9.84 JKL	374.04 ± 0.35 FGHI	406.54 ± 6.94 EFG	489.77 ± 6.35 BC	515.43 ± 11.08 AB	322.70 ± 6.84 JKL	297.09 ± 9.66 L	420.68 ± 5.81 DEF	386.60 ± 0.11 EFGH	309.03 ± 13.56 KL	359.57 ± 4.88 HIJ
Phosphorous	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	501.60 ± 1.29 ABC	459.69 ± 5.41 ABCDE	420.62 ± 1.98 EFG	460.49 ± 85.07 ABCDE	392.40 ± 1.87 EFGH	400.28 ± 2.04 EFGH	361.74 ± 4.80 GHI	506.66 ± 5.31 AB	497.70 ± 7.89 ABC	458.99 ± 2.15 ABCDE	346.44 ± 146.50 HI	436.64 ± 9.94 CDEF	421.35 ± 10.41 EFG
Steamed	524.12 ± 22.50 A	454.81 ± 14.10 ABCDE	394.05 ± 1.71 EFGH	384.15 ± 2.49 FGH	363.88 ± 1.36 GHI	360.08 ± 0.49 GHI	308.32 ± 0.47 I	517.50 ± 4.20 A	491.57 ± 0.57 ABCD	458.32 ± 1.79 ABCDE	437.72 ± 11.22 BCDEF	416.59 ± 7.81 EFG	426.96 ± 6.79 DEFG
Selenium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.08 ± 0.04 ABC	0.02 ± 0.01 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.04 ± 0.05 BC	0.01 ± 0.00 C	0.03 ± 0.03 C	0.12 ± 0.00 AB	0.06 ± 0.07 BC	0.01 ± 0.00 C	0.04 ± 0.05 BC
Steamed	0.02 ± 0.02 C	0.08 ± 0.10 ABC	0.02 ± 0.02 C	0.04 ± 0.04 BC	0.02 ± 0.01 C	0.02 ± 0.02 C	0.06 ± 0.01 BC	0.01 ± 0.00 C	0.03 ± 0.03 C	0.15 ± 0.03 A	0.08 ± 0.10 ABC	0.02 ± 0.01 C	0.05 ± 0.06 BC
Vanadium	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.04 ± 0.00 ABC	0.04 ± 0.00 AB	0.04 ± 0.00 ABCD	0.034 ± 0.00 BCDEFG	0.03 ± 0.00 CDEFGH	0.03 ± 0.00 BCDEFG	0.04 ± 0.00 ABCDEF	0.03 ± 0.01 EFGH	0.02 ± 0.00 H	0.02 ± 0.00 I	0.01 ± 0.00 I	0.03 ± 0.01 FGH	0.04 ± 0.00 ABCDE
Steamed	0.03 ± 0.01 CDEFGH	0.03 ± 0.00 FGH	0.04 ± 0.01 A	0.03 ± 0.00 GH	0.03 ± 0.00 GH	0.04 ± 0.00 ABCD	0.03 ± 0.00 CDEFGH	0.03 ± 0.00 DEFGH	0.03 ± 0.00 DEFGH	0.01 ± 0.00 I	0.01 ± 0.00 I	0.03 ± 0.00 BCDEFGH	0.04 ± 0.00 ABCDEF
Zinc	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	6.43 ± 0.03 ABCDE	5.70 ± 0.01 GHIJ	4.08 ± 0.08 KL	6.06 ± 0.89 CDEFG	5.78 ± 0.22 FGHI	5.53 ± 0.11 GHIJ	5.22 ± 0.04 IJ	6.91 ± 0.20 A	6.49 ± 0.13 ABCD	6.08 ± 0.18 CDEFG	6.48 ± 0.22 ABCD	6.29 ± 0.36 BCDEF	5.87 ± 0.08 EFGH
Steamed	6.28 ± 0.28 BCDEF	5.93 ± 0.27 DEFGH	4.35 ± 0.03 K	3.68 ± 0.28 L	5.40 ± 0.23 HIJ	5.14 ± 0.54 J	5.86 ± 0.07 FGH	6.63 ± 0.02 ABC	6.76 ± 0.45 AB	6.56 ± 0.00 ABC	5.76 ± 0.21 FGHI	5.53 ± 0.23 GHIJ	5.23 ± 0.12 IJ
Bismuth	**Day0**	**Day1**	**Day3**	**Day5**	**Day7**	**Day9**	**Day11**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Boiled	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.02 ± 0.01 B	0.01 ± 0.00 C	0.02 ± 0.01 B	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.02 ± 0.00 A	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C
Steamed	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C	0.01 ± 0.00 C

VITA

John Shackelford was born and raised in Baton Rouge, Louisiana. He attended Catholic High School of Baton Rouge and graduated in 2003. He then enrolled at Louisiana State University in Baton Rouge where he earned his bachelor's degree in biological sciences with a minor in English in December 2007. After working in upscale restaurants as a lead cook for four years, John decided to return to Louisiana State University to pursue a Master's degree in food science. John looks forward to a career in food research and product development.